CENTRE HOSPITALIER INTERCOMMUNAL
VILLENEUVE-SAINT-GEORGES
Lucie & Raymond AUBRAC

Observatoire des Médicaments Dispositifs médicaux Innovations Thérapeutiques

OMéDIT Centre-Val de Loire

LA PHAGOTHERAPIE EN 2017
UNE INNOVATION THERAPEUTIQUE !

Olivier PATEY

CH Lucie et Raymond AUBRAC

4ème journée des Référents OMEDIT Centre

21 septembre 2017

LesEchos.fr

LES ECHOS | Le 20/09 à 07:46

L'OMS s'alarme du manque de nouveaux antibiotiques

La résistance aux antimicrobiens est une urgence sanitaire qui met en péril les progrès de la médecine moderne, estime l'agence de santé des Nations unies.

Inquiétudes sur la tuberculose

L'OMS liste 12 « superbactéries » résistantes aux antibiotiques

ELEMENTS ABORDES

Phagothérapie : une thérapeutique antibactérienne découverte et utilisée depuis près de 100 ans

augmentation inquiétante des multirésistances bactériennes et des impasses thérapeutiques

Disparition de cette thérapeutique au début des années 1980 en Europe

Tentatives de réhabilitation de la phagothérapie depuis près de 20 ans

Nombreuses études d'efficacité in vitro et sur modèles animaux depuis 10 ans

Mais pratiquement aucune utilisation humaine à l'Ouest !
Nouveau scandale sanitaire du 21ème siècle ?

Publié le 26/04/2017 à 18:27

Un patient américain porteur d'une bactérie multirésistante sauvé grâce à la phagothérapie

Actu Santé

Aux Etats-Unis, les seuls phages commercialisés actuellement sont destinés à protéger les aliments contre des infections bactériennes. RelaxNews / Satirus / Istock.com

Un premier patient américain atteint d'une infection grave, ayant frôlé la mort, peut désormais retravailler après avoir reçu une phagothérapie, une thérapie antibactérienne utilisant des virus naturels, selon des médecins de l'école de médecine de San Diego aux Etats-Unis.

Tom Patterson, professeur de psychiatrie à l'université de San Diego (États-Unis), infecté par une bactérie naturellement résistante à de nombreux antibiotiques, appelée Acinetobacter baumannii, en 2015 lors d'un voyage en Égypte avec sa femme, est désormais sauvé, révèle le Pr Robert Schooley, chef du département des maladies infectieuses de l'école de médecine de San Diego et ami du malade.

C'est une thérapie alternative aux antibiotiques, nommée "phagothérapie" par un Franco-Canadien Félix d'Hérelle, microbiologiste à l'Institut Pasteur, qui a sauvé la vie à ce médecin de 71 ans.

Une fois rapatrié aux États-Unis quelques mois après être tombé malade, Tom Patterson a reçu, par intraveineuse directement dans l'abdomen, un cocktail de quatre virus mangeurs de bactéries, des "phages" collectés dans les eaux usées, terrain où ils se trouvent en grand nombre.

Il existe plusieurs milliards de milliards de ces "bactériophages". La recherche dans ce domaine n'a perduré principalement qu'en Europe de l'Est, et en particulier en Union soviétique (Pologne, Russie et surtout Géorgie) après les années 30, date de la mise au point des premiers antibiotiques.

Trois après jours après le début du traitement, le patient est sorti de son coma. Depuis juin 2016, la bactérie n'est plus présente dans son organisme.

Face à ce succès inattendu, l'équipe médicale de San Diego compte ouvrir un centre spécialement dédié à la recherche sur les bactériophages pour apporter des traitements personnalisés aux patients grâce à des combinaisons de phages.

Le centenaire de la recherche dans ce domaine est d'ailleurs célébré cette semaine à l'Institut Pasteur de Paris.

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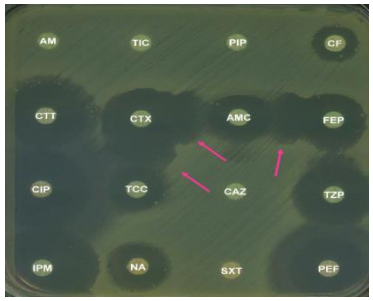
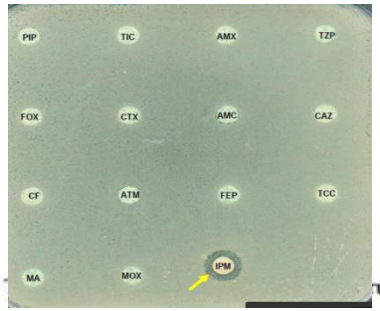
BMR
Bactérie **M**ulti**R**ésistante aux antibiotiques

BHR
Bactérie **H**autement **R**ésistante aux antibiotiques





Bactéries qui, du fait de l'accumulation des résistances naturelles et / ou acquises, ne sont plus sensibles qu'à un petit nombre d'antibiotiques habituellement actifs en thérapeutique

→ Réduction de l'arsenal thérapeutique

« Extensively Drug Resistant » (XDR) (consensus international)

Sensible à seulement 1 ou 2 classes d'antibiotiques

→ Impasse thérapeutique

ug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria

Magiorakos et al., CMI 2011

Bacterium	MDR	XDR	PDR
Staphylococcus aureus	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 1a*	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 1a.	Non-susceptibility to all agents in all antimicrobial categories for each bacterium in Tables 1a-1e
***Enterococcus* spp.**	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 1b	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 1b.	
Enterobacteriaceae	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 1c	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 1c.	
Pseudomonas aeruginosa	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 1d	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 1d.	
***Acinetobacter* spp.**	The isolate is non-susceptible to at least 1 agent in ≥ 3 antimicrobial categories listed in Table 1e	The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories in Table 1e.	

*All isolates are defined as MDR because resistance to oxacillin or cefoxitin predicts non-susceptibility to all categories of β-lactam antimicrobials listed in this document, with the exception of the anti-MRSA cephalosporins (i.e. all categories of penicillins, cephalosporins, β-lactamase inhibitors and carbapenems currently approved up until January 25, 2011).

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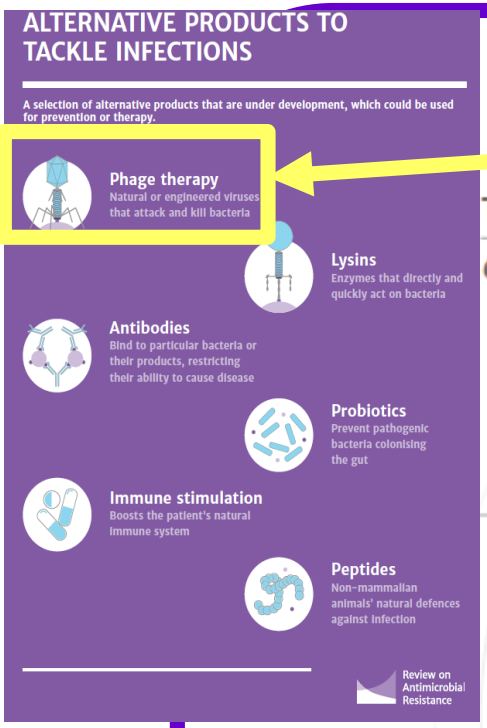
ESTIMATION DU NOMBRE DE DECES DANS LE MONDE

GEEPhage
Phagothérapie



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GEEPhage
Phagothérapie

LES DEUX PROTAGONISTES

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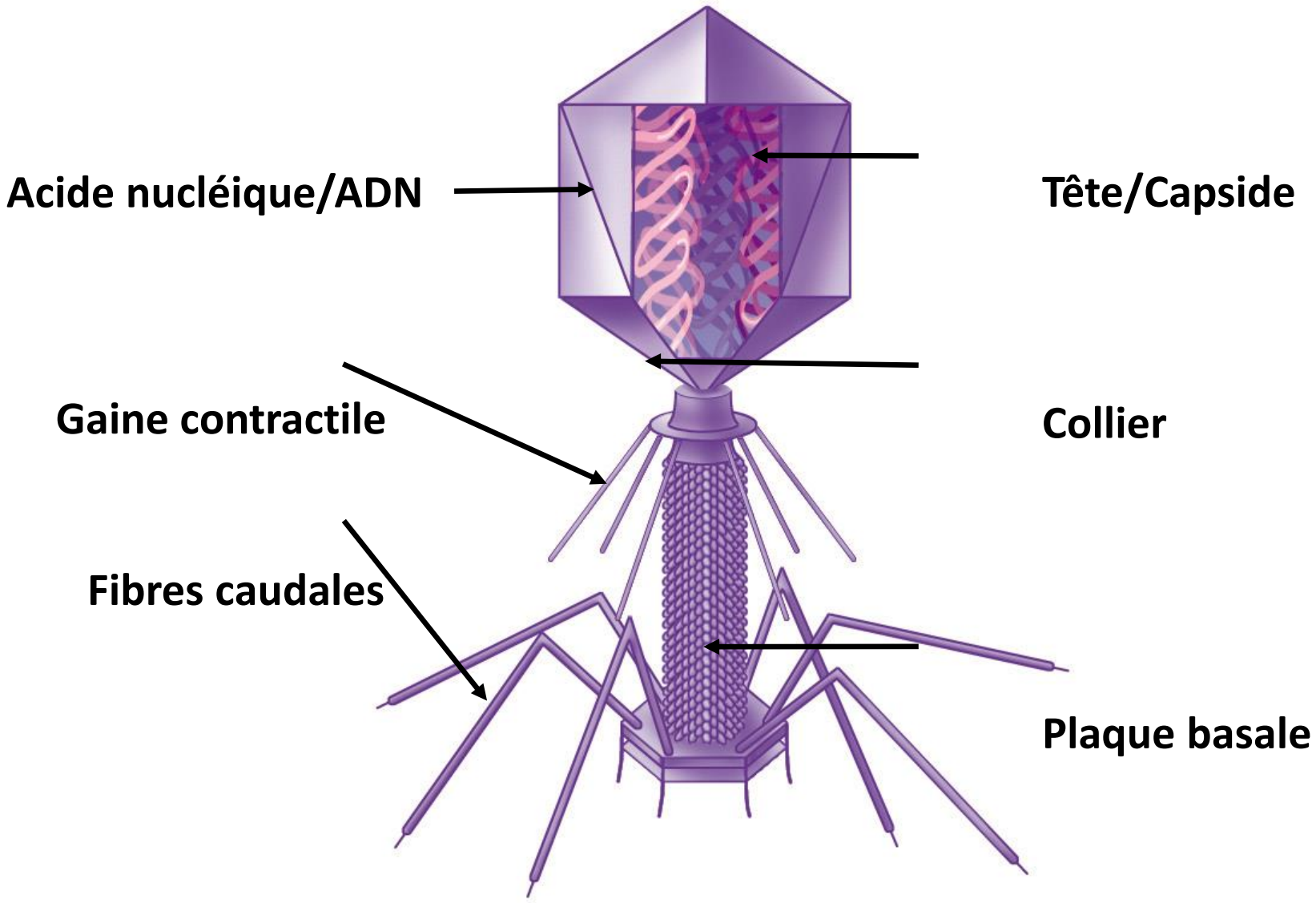
BACTERIOPHAGE T4

GEEPhage Phagothérapie



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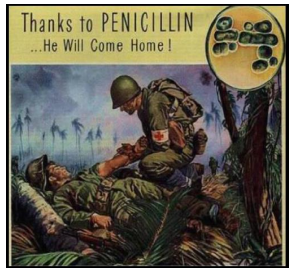
Bacteriophages characterized and named by d' Hérelle (1917)



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

Anciennes fioles contenant des phages à visée thérapeutique. [B]

al
ainst

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

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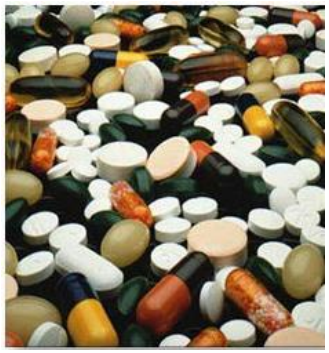

« Plus vous regardez loin en arrière,

Plus vous pourrez regarder loin en avant. »

Winston CHURCHILL

L'ACTION BACTÉRICIDE DES EAUX DE LA JUMNA ET DU GANGE SUR LE MICROBE DU CHOLÉRA

PAR M. E. HANKIN

Du laboratoire du gouvernement, Agra, Inde.

Quand on voit, à la traversée du Gange ou de la Jumna, au milieu d'une des grandes villes indiennes, des milliers d'habitants se laver, eux, leurs troupeaux et leurs vêtements dans une eau trouble et sale, et quand on songe que fréquemment des cadavres à moitié brûlés trouvent leur dernier asile dans le fleuve, on est bien excusable de penser que ces eaux doivent être dangereuses à consommer, et que la vénération des Hindous pour leur fleuve sacré prouve leur ignorance de toute idée de santé ou de propreté. C'est ce que pensent les autorités européennes, et, en ce qui concerne la distribution du

1. *Sur les microbes des rivières de l'Inde*. Communication au congrès médical indien tenu en décembre 1896.

Hankin published his observations in 1896 in the annals of the Institut Pasteur – that was the first evidence of the presence of bacteriophages in water and their antibacterial activities.

It was a viral-like agent with antibacterial properties. It was temperature sensitive and capable of passing through a porcelain filter, and it could reduce titres of the bacterium *Vibrio cholerae* in laboratory cultures.

"L'action bactericide des eaux de la Jumna et du Gange sur le vibrion du cholera„ *Annales de l'Institut Pasteur* (in French) 1896; 10: 511–523.

In 1915 The Lancet published an article written by Frederick Twort about “the transmissible bacterial lyses”. It was the first publication on bacteriophages.

stomach is explored manually up to the cardiac orifice, feeling for the induration around the perforated ulcer. Failing to find an ulcer on the anterior surface the stomach is pulled out with the transverse colon, and its posterior surface explored through an incision in the mesocolon.

A perforation is seldom of more than a quarter of an inch in diameter, though occasionally twice as large as this, and can be firmly occluded by the passage of one or two sutures. These sutures should secure a good wide grip through the whole thickness of the organ, since a small grip will easily tear out of the soft œdematous wall. The occluded ulcer should be invaginated where possible by a series of interrupted sutures taking up the serous and muscular coats. Invagination of the ulcer may, however, prove impossible if the ulcer and area of surrounding induration are very large, or in some instances where the ulcer is at the attachment of the duodenum to the posterior wall. In such cases the occluded ulcer is covered with a graft of detached omentum, or drainage is made down to the ulcer with a gauze pack (Corner[1]) in case the preliminary sutures cut out.

One must next consider whether a gastro-jejunostomy should be done. In most cases where the patient is not likely to die shortly we finish with a gastro-jejunostomy, especially where the ulcer is in the vicinity of the pylorus, since if this be done the patient can be fed after operation much more effectively, and there can be little doubt that many of these patients are suffering from malnutrition, the results of previous dyspepsia, which prevents healing taking place readily. This addition does not add greatly to the duration of the operation (the whole procedure from start to finish averages, we find, about 35 minutes) and improves the prospects of ultimate success. In the less usual cases where the ulcer is on the body of the stomach gastro-enterostomy is not so urgently needed, but nevertheless is advisable.

The Uses of Jejunostomy.

Where the patient's condition is extremely grave and every moment spent on the operation is of importance, we advise simply occluding the ulcer with one or two sutures, placing a gauze drain down to the site of perforation, and performing a jejunostomy for the purpose of feeding the patient early. Jejunostomy is performed on the invagination (Kader) principle, takes less than five minutes to perform, and has the advantage that fluid nourishment can be introduced to the most absorbent surface of the intestinal canal, in a situation where vomiting is impossible, and which, unlike the rectum, is unable to reject the proffered refreshment. The actual results of cases treated by this method were less good than were those of cases treated otherwise simply owing to the very grave condition of the patients; 1 recovered and 3 died. One of the latter, which had been perforated three days, lived four days after operation. Another lived

[1] THE LANCET, Jan. 10th, 1914, p. 101.

Suture and jejunostomy	1	...	2	.....	0 ... 1

My best thanks are due to my house surgeons for their notes on the above cases, and especially to Mr. W. S. Perrin, surgical registrar to the London Hospital, for his care in collecting and collating the histories.

Wimpole-street, W.

AN INVESTIGATION ON THE NATURE OF ULTRA-MICROSCOPIC VIRUSES.[1]

BY F. W. TWORT, L.R.C.P. LOND., M.R.C.S.

(From the Laboratories of the Brown Institution, London.)

DURING the past three years a considerable number of experiments have been carried out at the Brown Institution on filter-passing viruses. Many of these, previous to the outbreak of the war, were performed by Dr. C. O. Twort, and, unfortunately, circumstances during the present year have made it difficult to continue the work.

In the first instance attempts were made to demonstrate the presence of non-pathogenic filter-passing viruses. As is well known, in the case of ordinary bacteria for every pathogenic micro-organism discovered many non-pathogenic varieties of the same type have been found in nature, and it seems highly probable that the same rule will be found to hold good in the case of ultra-microscopic viruses. It is difficult, however, to obtain proof of their existence, as pathogenicity is the only evidence we have at the present time of the presence of an ultra-microscopic virus. On the other hand, it seems probable that if non-pathogenic varieties exist in nature these should be more easily cultivated than the pathogenic varieties; accordingly, attempts to cultivate these from such materials as soil, dung, grass, hay, straw, and water from ponds were made on specially prepared media. Several hundred media were tested. It is impossible to describe all these in detail, but generally agar, egg, or serum was used as a basis, and to these varying quantities of certain chemicals or extracts of fungi, seeds, &c., were added. The material to be tested for viruses was covered with water and incubated at 37° C. or over for varying periods of time, then passed through a Berkefeld filter, and the filtrate inoculated on the different media. In these experiments a few ordinary bacteria, especially sporing types, were often found to pass through the filter; but in no case was it possible to obtain a growth of a true filter-passing virus.

Attempts were also made to infect such animals as rabbits and guinea-pigs by inoculating two doses of the filtered material, or by rubbing this into the shaved skin. In other cases inoculations were made directly from one animal to another in the

[1] This investigation was made on behalf of the Local Government Board.

The Lancet, Jan. 10th, 1914, p. 101

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GEEPhage Phagothérapie

FELIX D'HERELLE

MICROBIOLOGIE. — *Sur un microbe invisible antagoniste des bacilles dysentériques*. Note (*) de M. **F. D'HERELLE**, présentée par M. Roux.

L'isolement du microbe anti-Shiga est simple : on ensemence un tube de bouillon avec quatre à cinq gouttes de selles, on place à l'étuve à 37° pendant 18 heures puis on filtre à la bougie Chamberland L_2. Une petite quantité d'un filtrat actif ajoutée, soit à une culture en bouillon de bacilles de Shiga, soit à une émulsion de ces bacilles dans du bouillon ou même dans de l'eau physiologique, provoque l'arrêt de la culture, la mort des bacilles puis leur lyse qui est complète après un laps de temps variant de quelques heures à quelques jours suivant l'abondance plus ou moins grande de la culture et la quantité de filtrat ajoutée.

Le microbe invisible cultive dans la culture lysée de Shiga car une trace de ce liquide, reportée dans une nouvelle culture de Shiga, reproduit le même phénomène avec la même intensité : j'ai effectué jusqu'à avec la première souche isolée, plus de 50 réensemencements su L'expérience suivante donne d'ailleurs la preuve visible que l'acti germe vivant : si l'on ajoute à une e culture précédente lysée, de façon enne qu'un millionième environ, et si sur gélose inclinée une gouttelette ubation, une couche de bacilles dyser re de cercles d'environ 1mm de diamèt s ne peuvent représenter que des col bstance chimique ne pourrait se conce it sur des quantités mesurées, j'ai pu vo

Anciennes fioles contenant des phages à visée thérapeutique. [B]

En résumé, chez certains convalescents de dysenterie, j'ai constaté que la disparition du bacille dysentérique coïncidait avec l'apparition d'un microbe invisible doué de propriétés antagonistes vis-à-vis du bacille pathogène. Ce microbe, véritable microbe d'immunité, est un bactériophage obligatoire; son parasitisme est strictement spécifique, mais s'il est limité à une espèce à un moment donné, il peut s'exercer tour à tour sur divers germes par accoutumance. Il semble donc que dans la dysenterie bacillaire, à côté d'une immunité antitonique homologue, émanant directement de l'organisme du sujet atteint, il existe une immunité antimicrobienne hétérologue produite par un microorganisme antagoniste. Il est probable que ce phénomène n'est pas spécial à la dysenterie, mais qu'il est d'un ordre plus général car j'ai pu constater des faits semblables, quoique moins accentués, dans deux cas de fièvre paratyphoïde.

D'Herelle F. C R Acad Sci Paris. 1917

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www.bacteriophage100.org

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PHAGOTHERAPIE 2020

First clinical application of the dysentery bacteriophage prepared by F. d'Hellere August 1, 1919, Paris

Victor-Henri Hutinel
1849-1933

Hôpital Necker – Enfants Malades supreetas
Assistance Publique – Hôpitaux de Paris

Essais de thérapeutique au moyen du Bactériophage du Staphylocoque,

par R. Bruynoghe et J. Maisin.

Nous avons eu l'occasion d'utiliser le Bactériophage du Staphylocoque dans un but thérapeutique et les quelques résultats favorables obtenus nous engagent à les signaler.

Cet essai nous semblait assez logique, étant donné que ces bactériolysats contiennent un principe immédiatement nuisible aux Staphylocoques et l'antigène staphylococcique indispensable à toute vaccination active dont les effets thérapeutiques ne peuvent se manifester que 5 ou 6 jours plus tard.

Dans notre note précédente nous avons vu que le sérum normal du Lapin n'empêche nullement le Bactériophage d'opérer la dissolution de ces microbes. Nous avons pu contrôler également ce fait pour le sérum humain. Dans ce but nous ensemençons des tubes de sérum stérile, d'un côté avec du Staphylocoque, d'un autre côté avec du Bactériophage et du Staphylocoque : l'inhibi-

tion de ces cultures déjà développées au moment d
Bactériophage.

Ce n'est pas ici la place de donner des détails cliniques concernant les malades que nous avons traités et nous nous contentons de signaler que nous avons appliqué cette thérapeutique chez 6 patients atteints d'anthrax ou de furoncles. Nous avons injecté aussi près que possible de la région malade, des doses de bactériolysats (stérilisées par une heure de chauffage à 56°) variant de 0,5 à 2 c.c. L'effet n'a pas tardé à se manifester par la diminution de l'empâtement au niveau des lésions et souvent par la disparition totale de ces dernières en 24 à 48 heures. Les infections déjà arrivées à la suppuration se vident et sèchent rapidement.

A la suite de ces inoculations, il se produit, chez certains malades, une ascension fébrile, chez d'autres la température ne subit guère de modification. Il nous a semblé que cette élévation de la température se produit surtout chez ceux atteints de vastes lésions et où la lyse rapide entraîne la résorption de grandes quantités de produits microbiens. L'endroit d'injection est durant 24 heures douloureux et légèrement œdématié.

Nous avons essayé cette thérapeutique chez des patients atteints d'anthrax ou de furoncles, mais il n'est pas impossible que d'autres lésions telles que les acnés et les diverses complications staphylococciques d'autres affections cutanées ne puissent profiter de la même médication.

Ces observations ne sont évidemment pas assez nombreuses pour établir définitivement la valeur de cette méthode et elles n'ont pu être assez prolongées pour déterminer jusqu'à quel point ces inoculations protègent contre les rechutes.

(*Institut de bactériologie de l'Université de Louvain*).

Bruynoghe R, Maisin J. C R Soc Biol. 1921

BULLETIN OF
THE NEW YORK
ACADEMY OF MEDICINE

VOL. VII MAY, 1931 No. 5

ANNUAL GRADUATE FORTNIGHT

BACTERIOPHAGE AS A TREATMENT IN ACUTE MEDICAL AND SURGICAL INFECTIONS*

F. d'Herelle
Professor of Bacteriology,
Yale University School of Medicine

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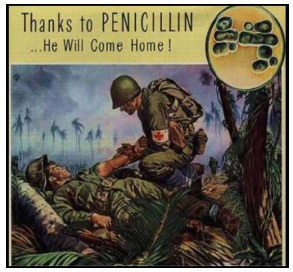

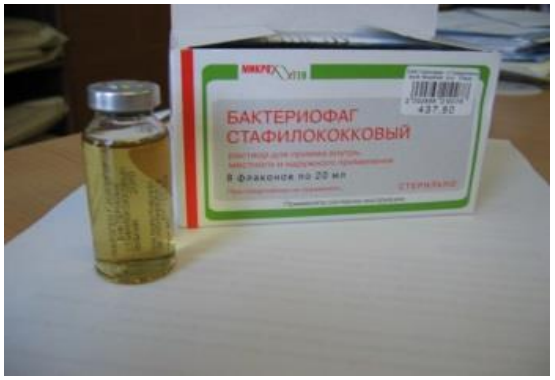

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Phage genome sequencing begins (1980s) and continues to present day

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ELIAVA INSTITUTE TBILISSI, GEORGIA

George Eliava and Felix D'Herelle at one of the conferences held in the Soviet Union in 1930's.

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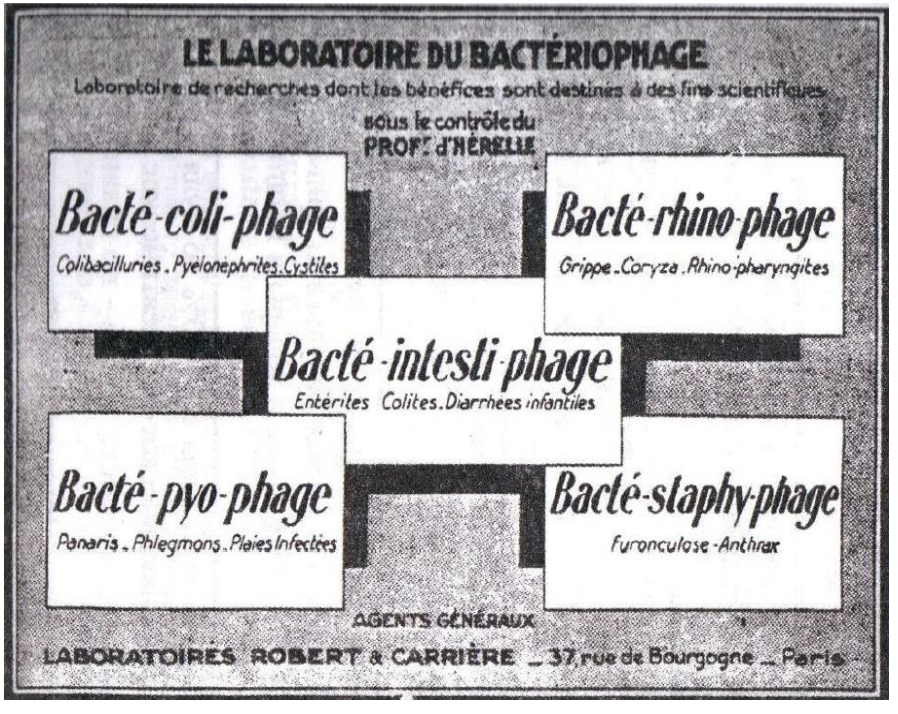
Bacteriophage producers in the USSR in 1930s-1940s

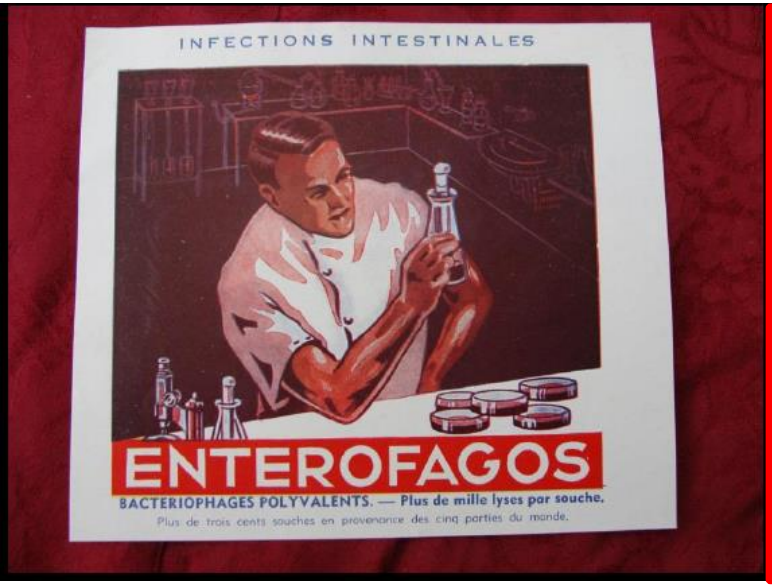
Krestovnikova V.A. (1947) Phage therapy and phage prophylaxis and their development through the work of Soviet researchers

- Mechnikov Research Institute of Vaccines and Sera, Moscow
- Leningrad Research Institute of Vaccines and Sera
- Tbilisi Research Institute of Vaccines and Sera
- Mechnikov Research Institute of Vaccines and Sera, Ufa
- Perm Research Institute of Vaccines and Sera
- Gorky Research Institute of Vaccines and Sera
- Ukrainian Research Institute of Epidemiology and Microbiology
- Mechnikov Research Institute of Vaccines and Sera, Kharkiv
- Khabarovsk Research Institute of Epidemiology and Microbiology
- Tashkent Research Institute of Vaccines and Sera
- Tomsk Research Institute of Vaccines and Sera
- Kazakhstan Research Institute of Epidemiology and Microbiology



WWII:
Red Army (USSR)
German Army (North Africa campaign)
Japanese Arm

Au sein de l'Union soviétique et des forces de l'Axe, les bactériophages ne furent pas mis tout de suite de côté, l'antibiothérapie n'étant au début de la seconde guerre mondiale que balbutiante. Allemands, Japonais et Soviétiques continuèrent de soigner les infections avec des phages. Le paquetage militaire distribué aux soldats de la Wehrmacht contenait même des flacons de suspensions de phages à appliquer sur les blessures de guerre. Les médecins allemands utilisèrent en particulier un traitement contre la dysenterie, nommé « Typhus-Paratyphus B-Polyfagin » (figure 8). Lorsqu'une partie de la France fut occupée, les troupes d'occupation réquisitionnèrent le « Laboratoire du Bactériophage » pour produire des préparations phagiques (ils n'exploitèrent cependant que les installations, et non pas les produits phagiques déjà créés par le laboratoire) pour ravitailler leurs troupes. D'Hérelle dut



Schachtel mit 3 Amp. zu 1 ccm 1,40
Flasche zu 10 cm . . . . . —,70

17 **Typhus-Paratyphus B-Polyfagin (Bakteriophagen Behringwerke)**
Zus. Polyvalente Vaccine mit 500 Mill. Typhus- u. je 250 Mill. Paratyphus A- u. B-Bazillen pro ccm, 0,5% Phenol.
Ind. Prophylaxe des Typhus und Paratyphus.
Dos. 2—3 × 0,5 bzw. 1 ccm subkutan in Abständen von 7—8 Tagen.
Packung A (orale Anwendung):
Schachtel mit 6 Amp. zu 10 ccm 4,65

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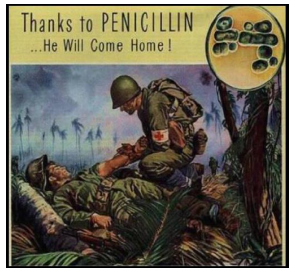
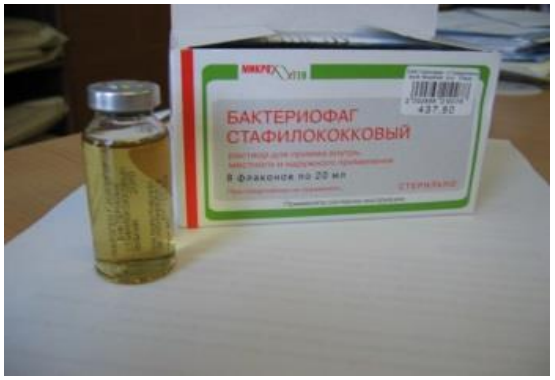
GEEPhage
Phagothérapie

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

Anciennes fioles contenant des phages à visée thérapeutique. [B]

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)

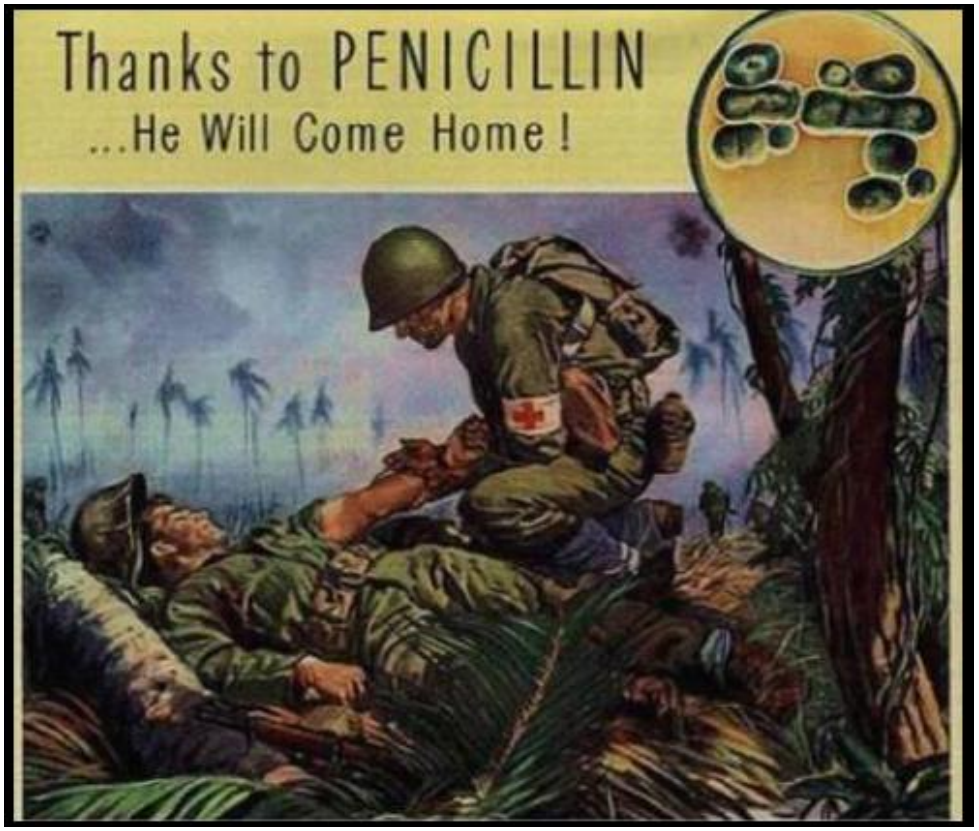
Fischetti' s group demonstrate in vivo activity of phage lysins (2001)

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.

LE TUEUR DU BACTERIOPHAGE



PHAGOTHERAPIE ET CHOLERA

In late 1960s, the World Health Organization (WHO) set up an international trial of phage therapy for cholera in Dhaka. This trial was designed according to the widely accepted international standards and conducted with the support and under the review of the National Institutes of Health, USA. In several WHO sponsored studies in East Pakistan (now Bangladesh) in the 1970s, bacteriophage therapy was compared with tetracycline as the therapeutic agent. It was reported that very high dose phage therapy was comparable to tetracycline in reducing the excretion of vibrios in the stool[17, 18].

17. Monsur, K.A., Rahman, M.A., Huq, F., Islam, M.N., Northrup, R.S. and Hirschhorn, N. Effect of massive doses of bacteriophage on excretion of vibrio, duration of diarrhoea and output of stools in acute cases of cholera. *Bull World Health Organ 42:* 723, 1970.

18. Marcuk, L.M., Nikiforov, V.N., Scerbak, J.F., Lovitov, T.A., Kotljarova, R.I., Mammsina, M.S., Dovydov, S.U., Monsur, K.A., Rahman, M.A., Latif, M.A., Northrup, R.S., Cash, R.A., Huq, I., Dey, C. and Phillips RA. Clinical studies of the use of bacteriophage in the treatment of cholera. *Bull World Health Organ 45:* 77, 1971.

utilisation mondiale

- Eli Lilly and Company
- Swan Myers (Abbot Laboratories)
- Squibb and sons (B. M. S.)
- Laboratoire Parke-Davis (Pfizer)
- Instituts Pasteur de Paris et de Lyon
- Laboratoire du Bactériophage (5 rep.) (Robert & Carrière)
- German company Antipiol
- Saphal en Suisse
- IP Paris et Lyon
- Géorgie –Russie
- Delmont Laboratories aux USA

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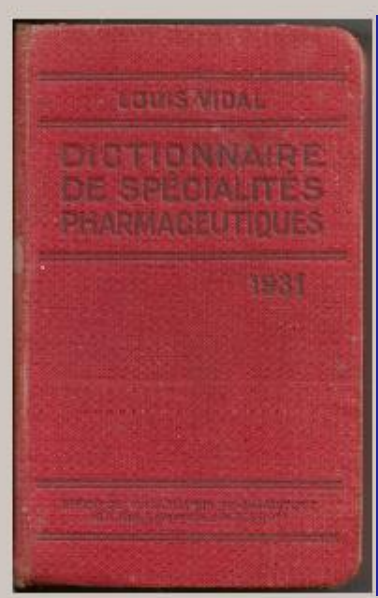
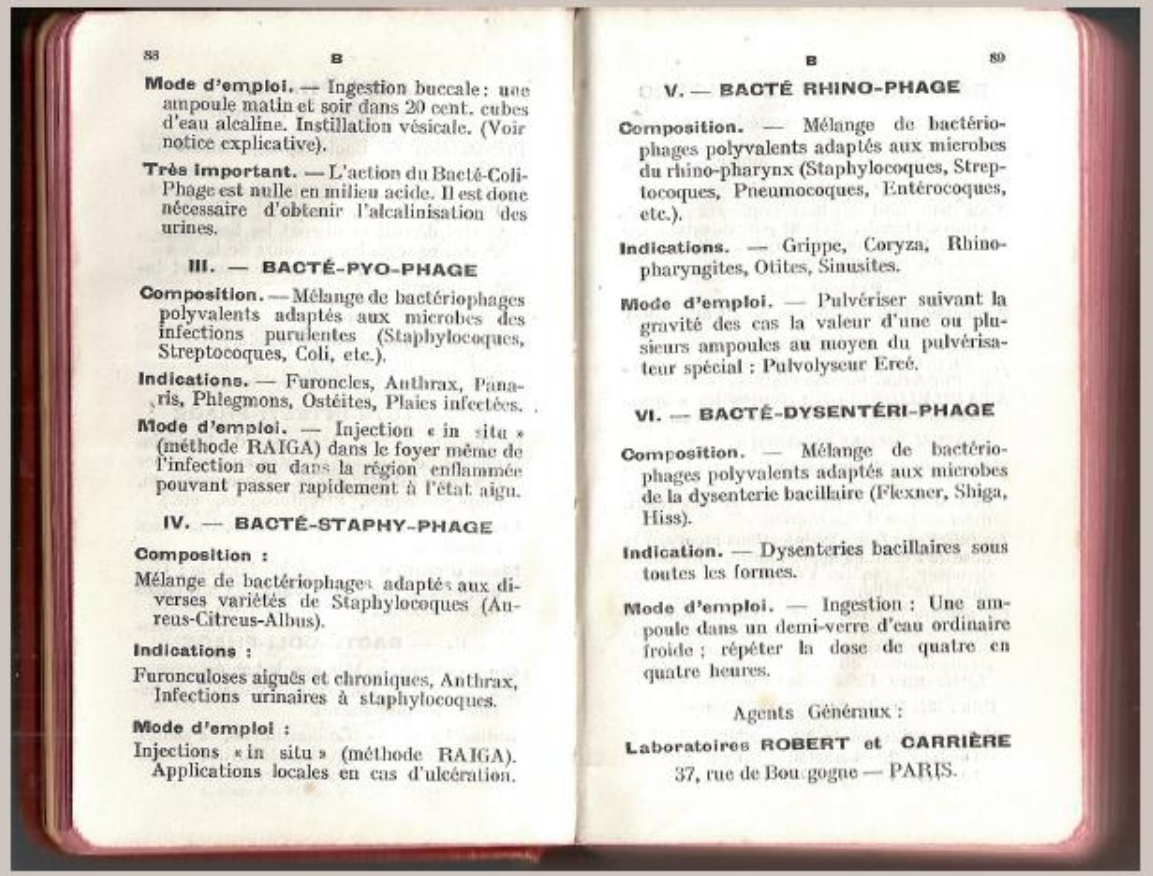


- dernière commercialisation 1974 (Vidal)

  Laboratoires Robert & Carrière
- dernières disponibilités officielles 1990 (I.P.)

  IP de Paris (J.-F.Vieu) et IP de Lyon (F. Guillermet)
- aujourd'hui pas interdite…

…mais pas autorisée !



88 B

Mode d'emploi. — Ingestion buccale : une ampoule matin et soir dans 20 cent. cubes d'eau alcaline. Instillation vésicale. (Voir notice explicative).

Très important. — L'action du Bacté-Coli-Phage est nulle en milieu acide. Il est donc nécessaire d'obtenir l'alcalinisation des urines.

III. — BACTÉ-PYO-PHAGE

Composition. — Mélange de bactériophages polyvalents adaptés aux microbes des infections purulentes (Staphylocoques, Streptocoques, Coli, etc.).

Indications. — Furoncles, Anthrax, Panaris, Phlegmons, Ostéites, Plaies infectées.

Mode d'emploi. — Injection « in situ » (méthode RAIGA) dans le foyer même de l'infection ou dans la région enflammée pouvant passer rapidement à l'état aigu.

IV. — BACTÉ-STAPHY-PHAGE

Composition :

Mélange de bactériophages adaptés aux diverses variétés de Staphylocoques (Aureus-Citreus-Albus).

Indications :

Furonculoses aiguës et chroniques, Anthrax, Infections urinaires à staphylocoques.

Mode d'emploi :

Injections « in situ » (méthode RAIGA). Applications locales en cas d'ulcération.

B 89

V. — BACTÉ RHINO-PHAGE

Composition. — Mélange de bactériophages polyvalents adaptés aux microbes du rhino-pharynx (Staphylocoques, Streptocoques, Pneumocoques, Entérocoques, etc.).

Indications. — Grippe, Coryza, Rhino-pharyngites, Otites, Sinusites.

Mode d'emploi. — Pulvériser suivant la gravité des cas la valeur d'une ou plusieurs ampoules au moyen du pulvérisateur spécial : Pulvolyseur Ereé.

VI. — BACTÉ-DYSENTÉRI-PHAGE

Composition. — Mélange de bactériophages polyvalents adaptés aux microbes de la dysenterie bacillaire (Flexner, Shiga, Hiss).

Indication. — Dysenteries bacillaires sous toutes les formes.

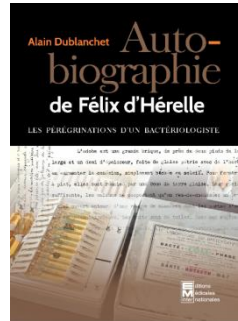
Mode d'emploi. — Ingestion : Une ampoule dans un demi-verre d'eau ordinaire froide ; répéter la dose de quatre en quatre heures.

Agents Généraux :

Laboratoires ROBERT et CARRIÈRE

37, rue de Bourgogne — PARIS.

36. Dictionnaire des spécialités pharmaceutiques Louis Vidal de 1931.



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.

Pétition en faveur de la renaissance de la bactériophagie thérapeutique

Frappé par le désarroi des malades se trouvant, depuis plusieurs mois, dans l'impossibilité de trouver en pharmacie les ampoules de Bactériophage qui leur avaient été prescrites ou bien de renouveler la provision que les plus prévoyants d'entre eux avaient constituée pour surmonter victorieusement, dès le prime début, tout assaut microbien, j'ai pensé que la Société des Amis de Félix d'Hérelle se devait de mettre la puissance de son autorité au service de ces doléances qui m'étaient exprimées pour contribuer à redonner à la Phagothérapie tous les moyens d'expression dont la prestigieuse découverte du Pr d'Hérelle l'a dotée. Evidemment, le thérapeute peut toujours, car aucune force au monde ne peut empêcher qu'un phénomène naturel existe, préparer son malade pour faciliter chez lui, grâce à son propre bactériophage intestinal, la production naturelle de la guérison spontanée des maladies infectieuses. Mais cette « guérison naturelle dirigée » a besoin, pour parfaire son action salvatrice, du ré-ensemencement du milieu intestinal par des races nouvelles et virulentes de Bactériophage. Ce sont ces races que le Laboratoire, qui a cessé son activité pour des raisons qui me sont inconnues, sélectionnait sous forme des préparations distribuées à la clientèle par les pharmacies.

J'ai donc résolu, en vue de constituer une véritable pétition à présenter en haut lieu, à convier :

— Vous, mes maîtres, mes collègues, et mes confrères qui êtes devenus, pour le plus grand bien de vos malades, les adeptes du Bactériophage de d'Hérelle ;

— Vous, malades ou blessés qui avez bénéficié, aussi bien sur vous-mêmes que sur vos proches ou vos amis, des succès remportés au cours des applications thérapeutiques du Phénomène de Bactériophagie ;

— Vous, membres de la société des amis de Félix d'Hérelle qui vous êtes groupés pour pouvoir, par le soutien de chacun, assurer la pérennité de l'œuvre exceptionnelle de ce grand savant qui, venu de Montréal, a offert à la France la gloire attachée à la découverte d'un phénomène naturel dont le microscope électronique, fait unique dans la Science, a confirmé dans ses moindres détails la réalité que la méthode expérimentale seule avait permis de dévoiler :

à signer une attestation pour influencer par le nombre des signataires les milieux scientifiques aptes à relever le flambeau qui est tombé par suite de la défaillance du Laboratoire qui avait été fondé par d'Hérelle en 1928 et qui a permis, au cours des 50 années qui suivirent, la mise au point d'un traitement d'une efficacité jamais égalée par sa qualité, sa constance et sa parfaite innocuité : la Phagothérapie. Cette défaillance est d'autant plus catastrophique que, d'une part, ce Laboratoire était le seul à assurer la préparation des différentes races de Bactériophage qui sont indispensables à la pratique médicale et chirurgicale dans le domaine de toute la pathologie infectieuse et que, d'autre part, le phénomène de Bactériophagie qui est reproduit dans un but thérapeutique ne peut être remplacé par aucune spécialité pharmaceutique puisque, seul de tous les traitements, il est l'expression d'un phénomène de la nature. L'arrêt de ce Laboratoire a pour conséquence inéluctable d'entraîner dans sa chute la pratique d'une méthode thérapeutique qui a fait ses preuves dans tous les domaines depuis 1917, date à laquelle le phénomène de Bactériophagie fut révélé au monde du haut de la tribune de l'Académie des Sciences.

Félix d'Hérelle.

C'est donc un devoir pour la Société des amis de Félix d'Hérelle, dont la mission est d'assurer la défense de l'œuvre du Pr d'Hérelle contre toute manœuvre dirigée directement ou indirectement contre elle, que d'appuyer de son influence toutes initiatives généreuses qui sont disposées à œuvrer dans ce sens. Plus les voix seront nombreuses, plus elles auront chance d'être entendues.

Disciples convaincus de la Bactériophagie, venez tous à nous en nous offrant le poids de votre signature.

Dr A. Raiga-Clémenceau

Pour tous renseignements, s'adresser au Dr Raiga-Clémenceau, 11, rue Boissière, 75116 Paris.

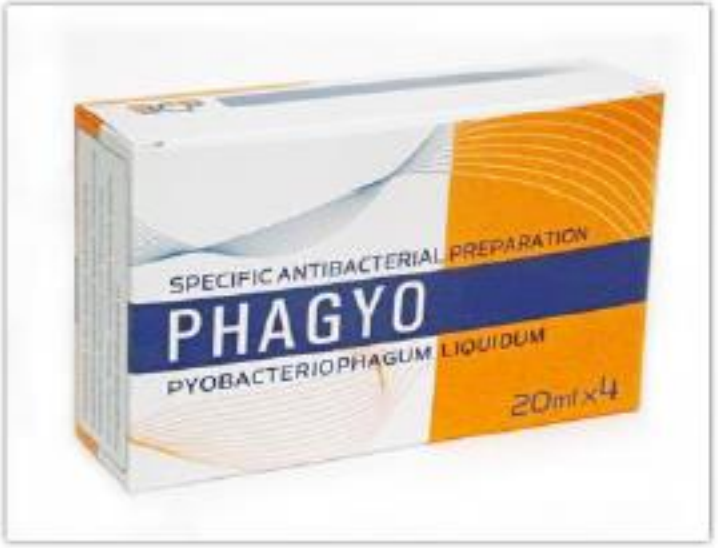
Journées Nationales d'Infectiologie

Jeudi **14** et Vendredi **15** juin 2001

Nantes

Cit des Congrès

et la région Centre-Ouest



Vendredi 15 juin 2001

Salle 200 : 11 h 00 à 12 h 30

CP 4

Session de communications orales en partenariat

avec le Contrôle Epidemiologique des Maladies Infectieuses (CEMI)

Coordinateur : O. Patey

Thème : Actualités sur les phages

Modérateurs : Ch. Lafaix, M. Vieux

CP4-02 *11h 05* Historique.
H. de Montclos.

CP4-03 *11h 15* Taxonomie.
H.W. Ackerman.

CP4-04 *11h 25* Le phage comme vecteur de gènes.
L. Bossi.

CP4-05 *11h 35* Mesures de contamination des eaux.
C. Gantzer.

CP4-06 *11h 45* Place actuelle dans le diagnostic et en épidémiologie.
F. Grimont.

CP4-07 *11h 55* Phagothérapie.
N. Chanishvili.

CP4-09 *12h 15* Discussion - Conclusion.
A. Dublanchet.

Vendredi 15 juin 2001
matinée

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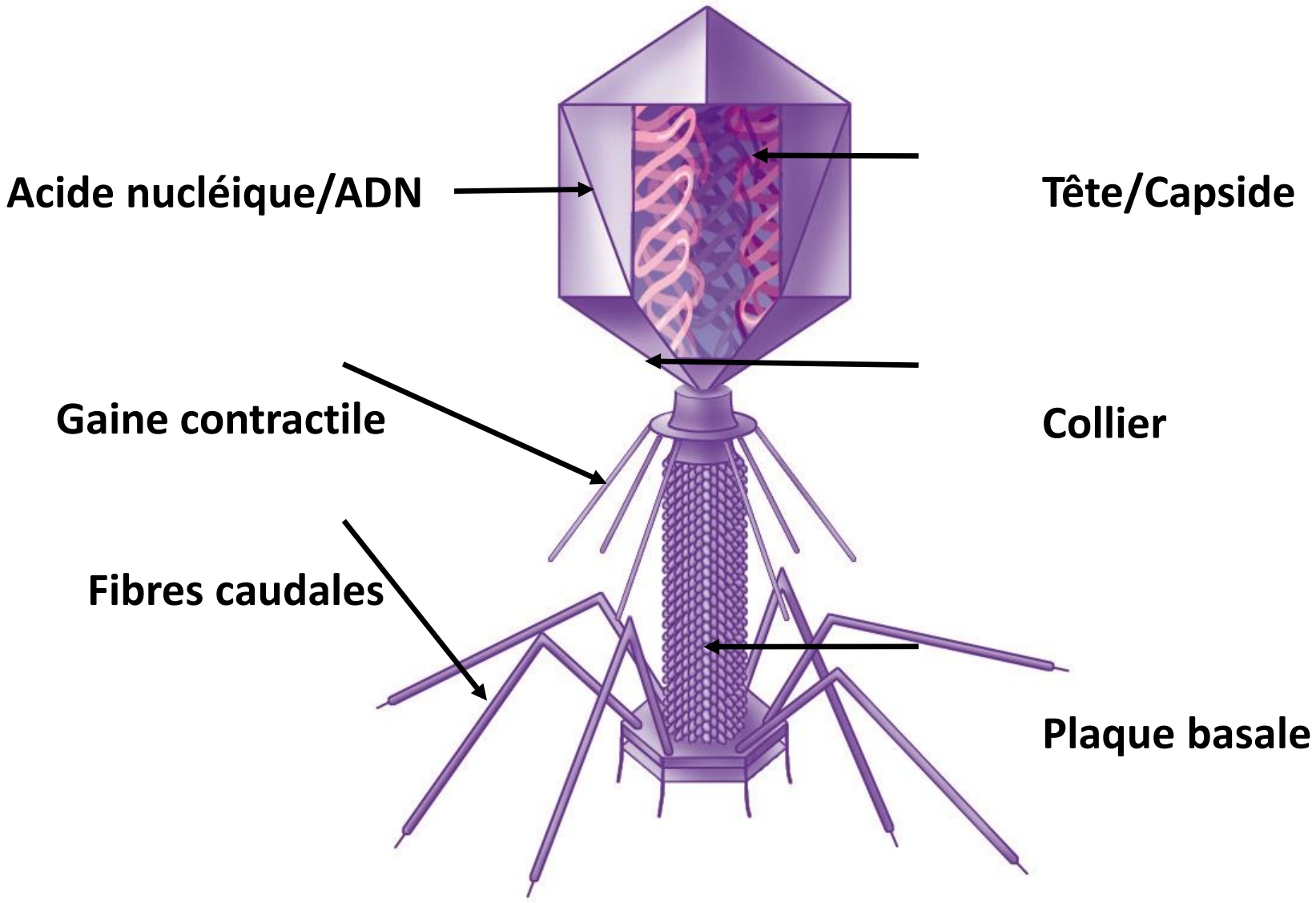
BACTERIOPHAGE T4

GEEPhage Phagothérapie



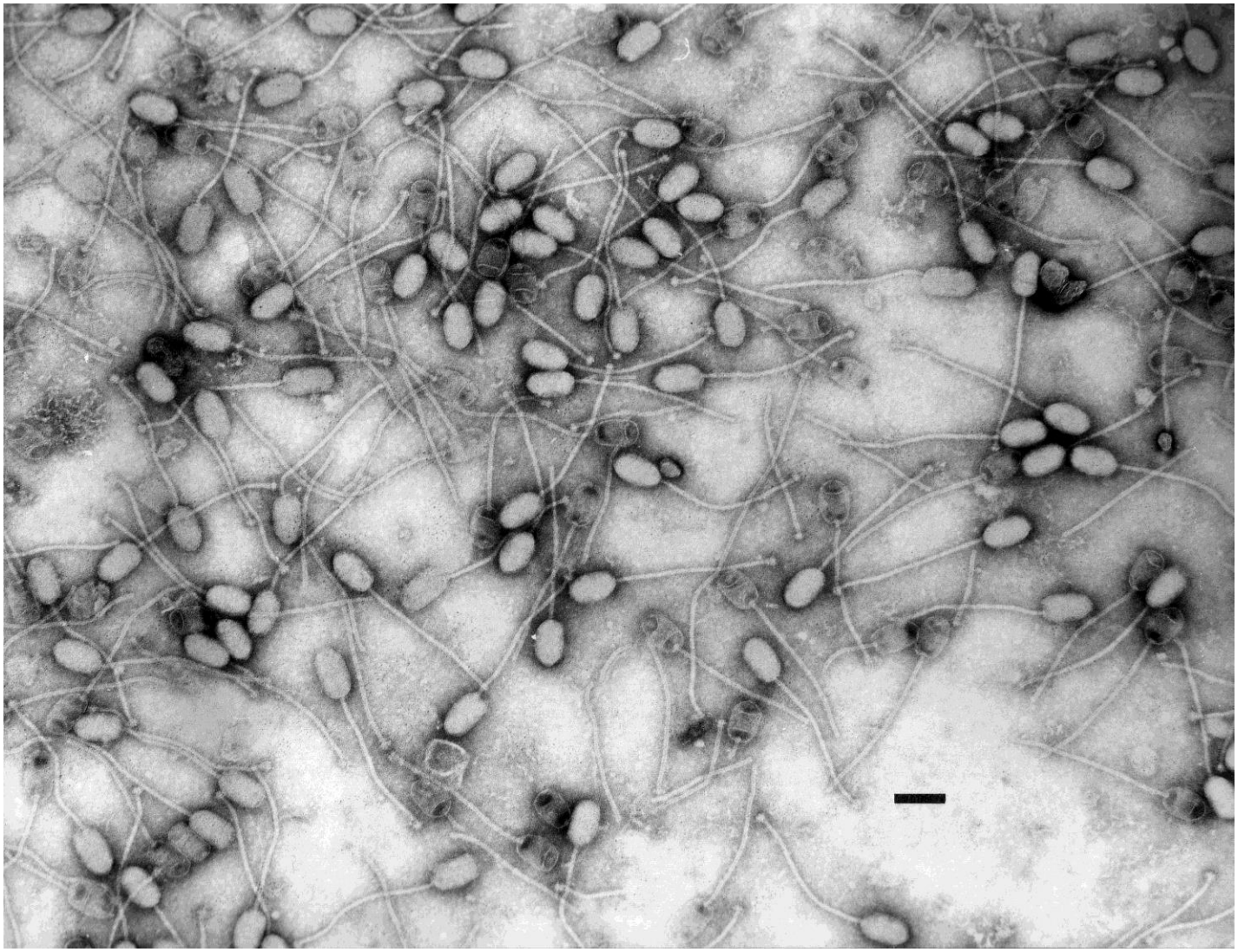


Photo 1 : Bactériophage 3A de Staphylococcus aureus

(Microscopie éléctronique ; coloration : acétate d'uranyl ; grossissement : x 92 400)

Cliché H.W. Ackermann

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GEEPhage Phagothérapie

CLASSIFICATION DES BACTERIOPHAGES

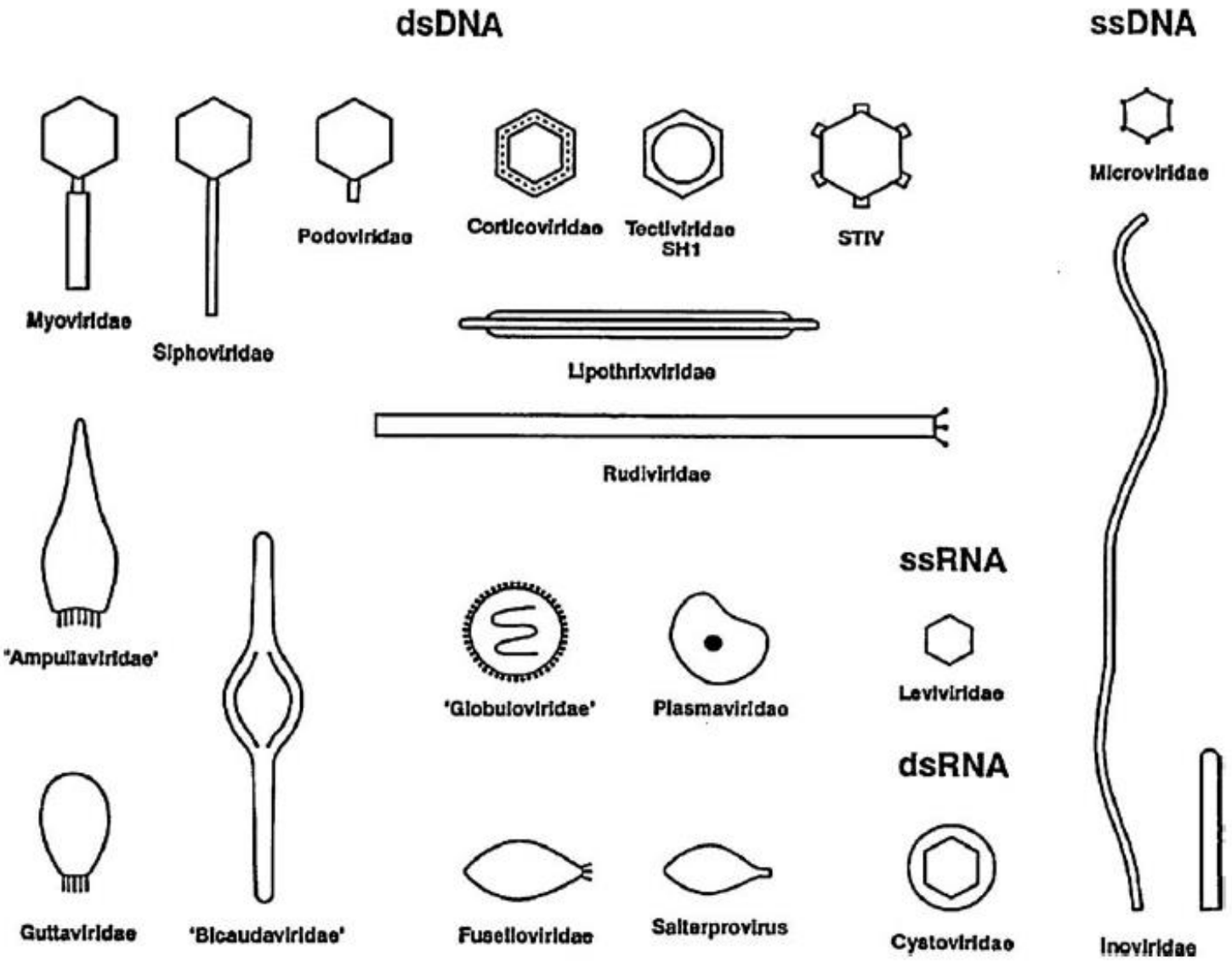

Figure 2 Morphologie des principaux groupes de bactériophages. D'après et avec autorisation de H.W. Ackermann [77].

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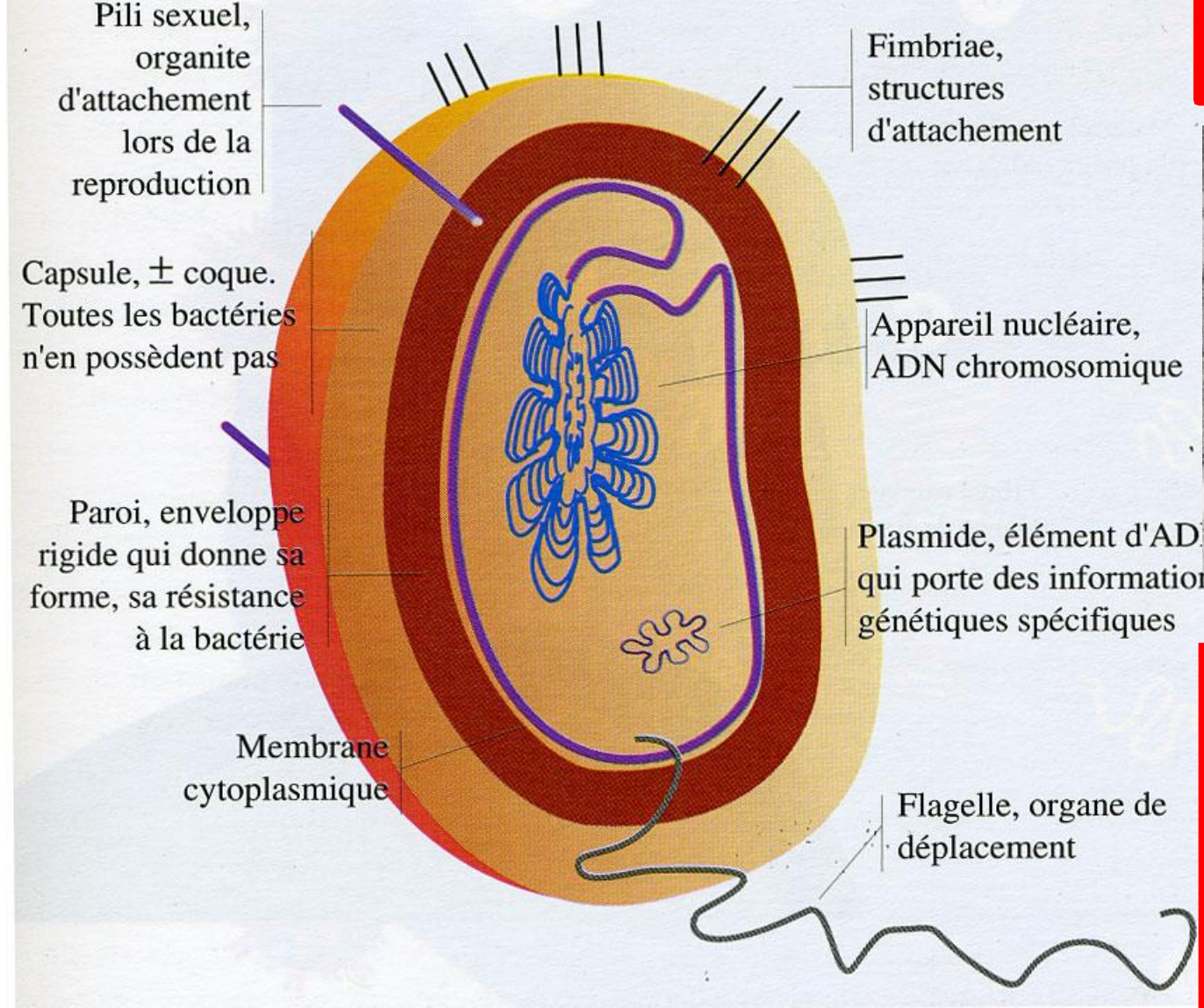
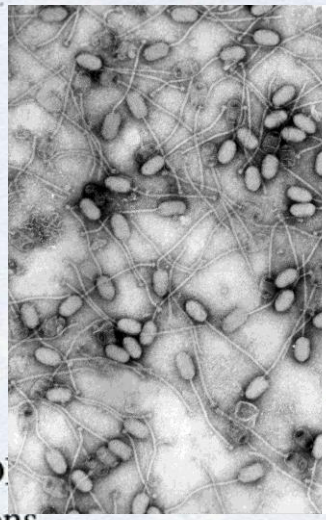
PHAGE ET BACTERIE

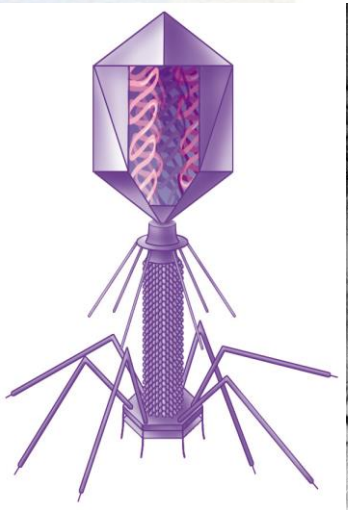
GEEPhage
Phagothérapie

BACTÉRIE
(Taille moyenne : 1 000 nanomètres)



BACTÉRIOPHAGE
(50 nm)





10^{32} bactériophages
(<10% connus)
(< 6 000 φ identifiés)

10^{31} bactéries (20% détruites/24H)

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GEEPhage
Phagothérapie

ASPECTS QUANTITATIFS

Bacteriophages live in soil (about 10^8 phages per 1 g soil); in rivers, lakes, and oceans (10^6 phages per 1 ml of sea water); and in other living beings (the human gastrointestinal tract contains about 10^{12} phages). As for the total bacteriophage population, it reaches according to scientific estimates an astronomic value of 10^{31} phages, or 10^9 tons of substance. This peculiar "dark matter" of biological origin spans the overall earth biosphere.

Some more statistics: assuming that an average diameter of a phage particle is 50 nm, all bacteriophages of the globe put in line will cover a distance of 5×10^{20} kilometers or 5×10^7 light years! This is the distance to Virgo Supercluster, nearest to us, while only 4.2 light years separate us from the nearest star, the Proxima Centauri

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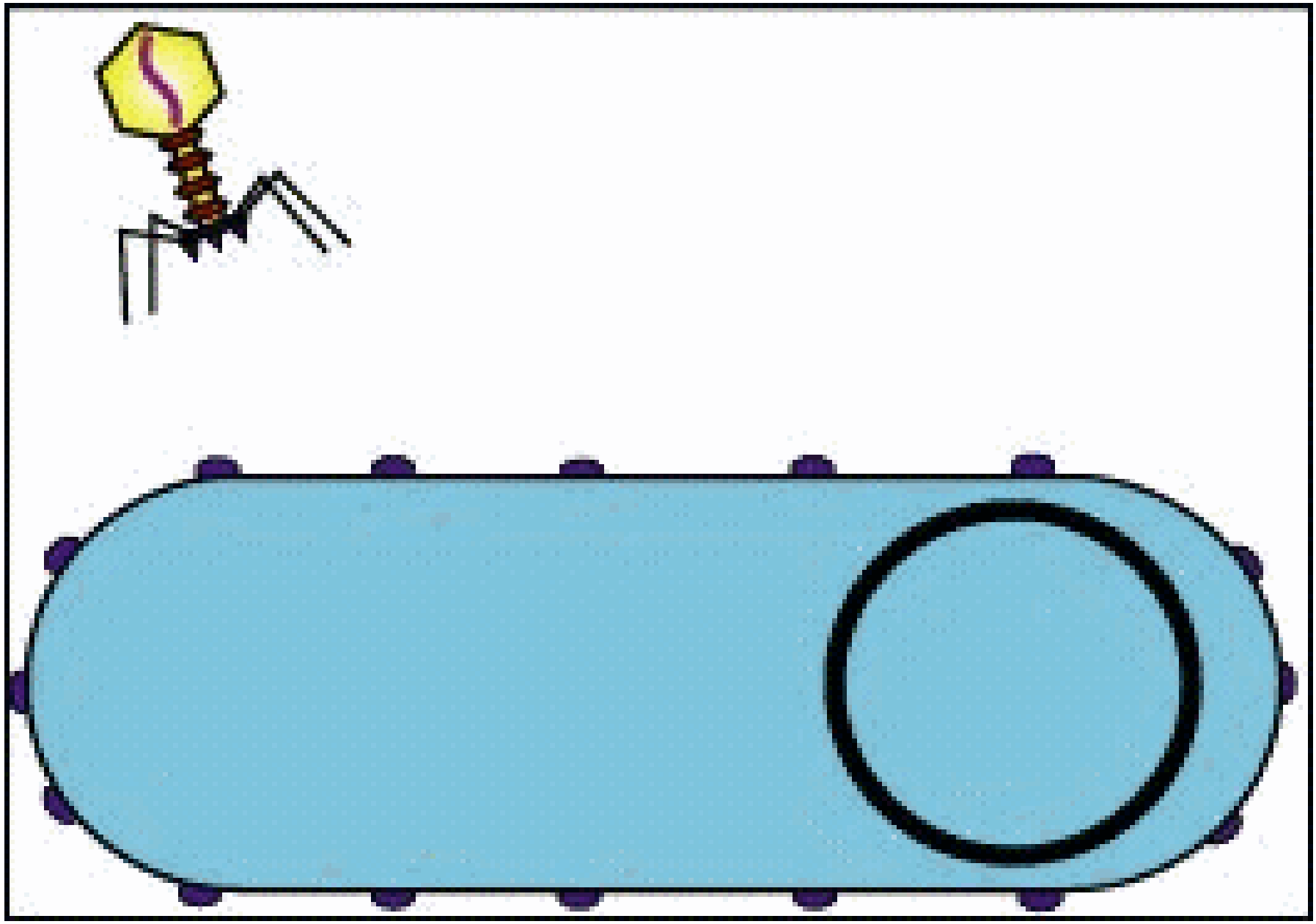
GEEPhage
Phagothérapie

CYCLE LYTIQUE



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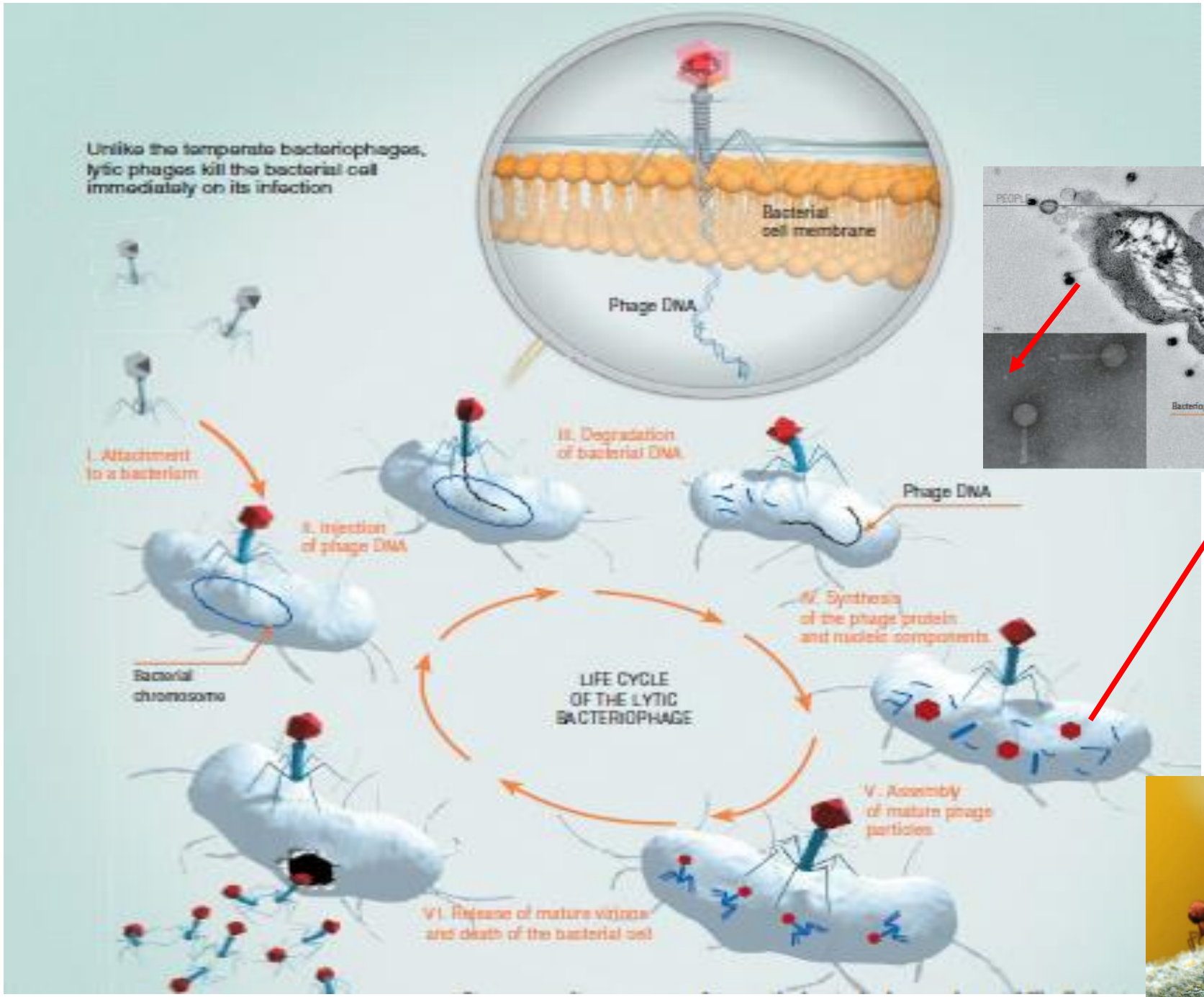
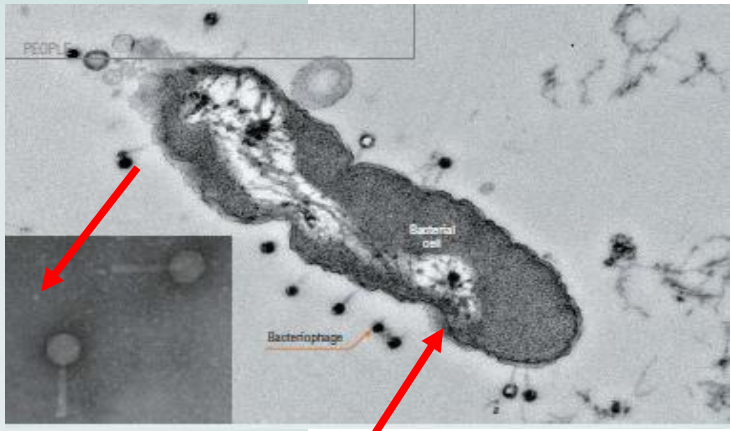
CYCLE LYTIQUE

GEEPhage
Phagothérapie





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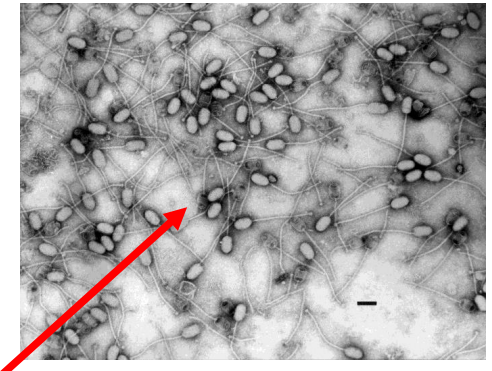
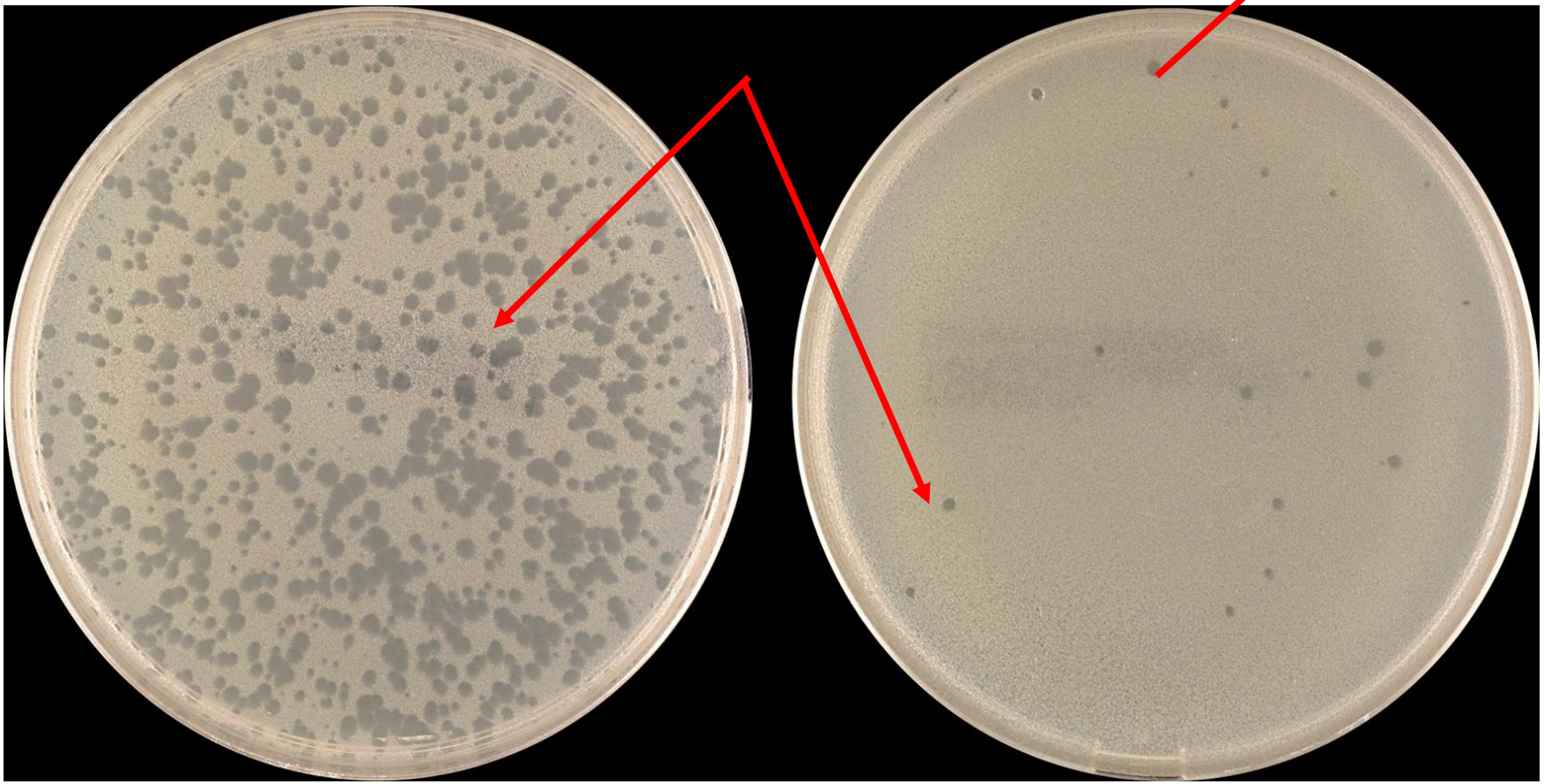
GEEPhage
Phagothérapie

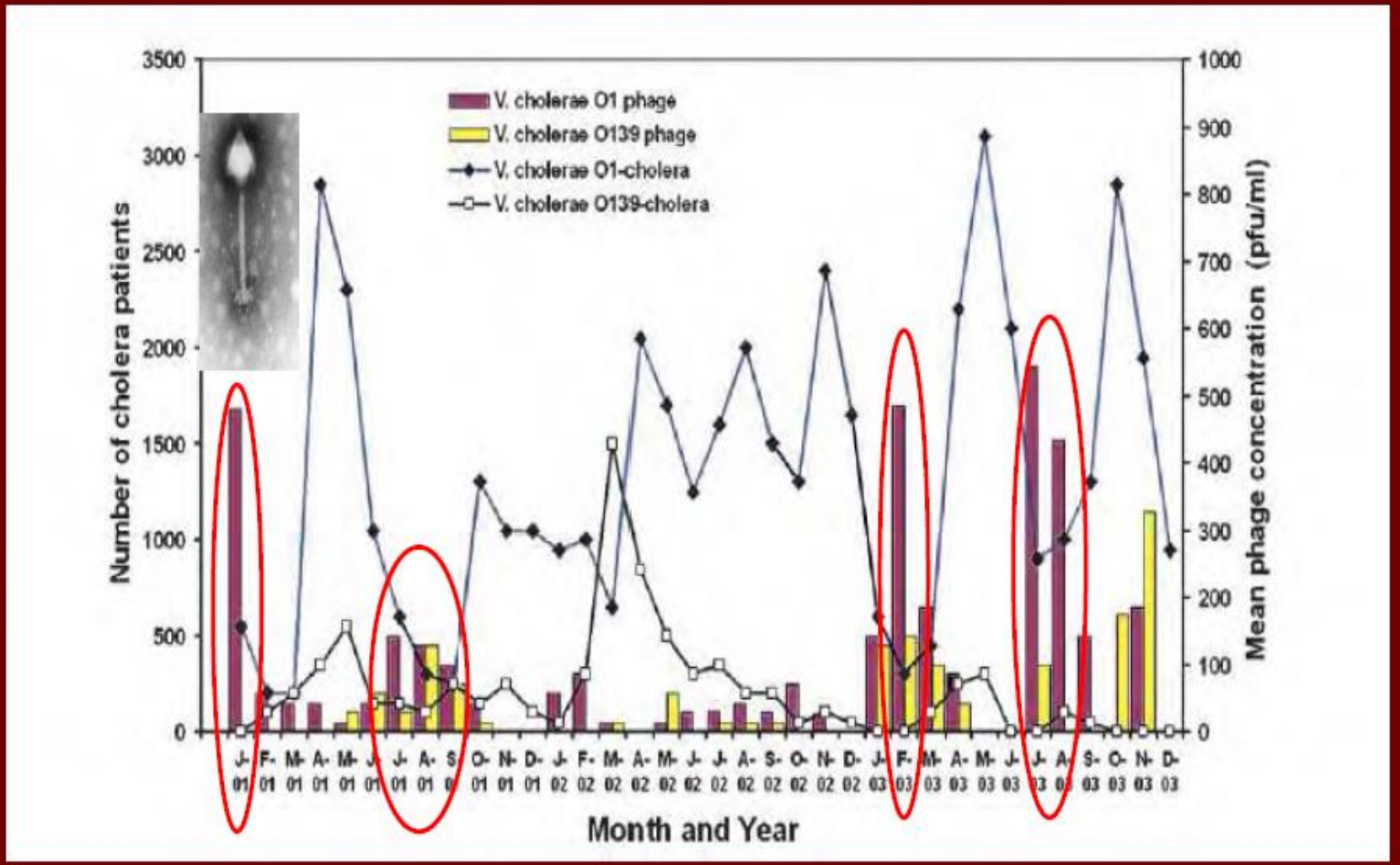
PHAGE COUNT





Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages

Shah M. Faruque*, Iftekhar Bin Naser*, M. Johirul Islam*, A. S. G. Faruque*, A. N. Ghosh†, G. Balakrish Nair*, David A. Sack*, and John J. Mekalanos‡§



Faruque. PNAS. 2004: 1-6

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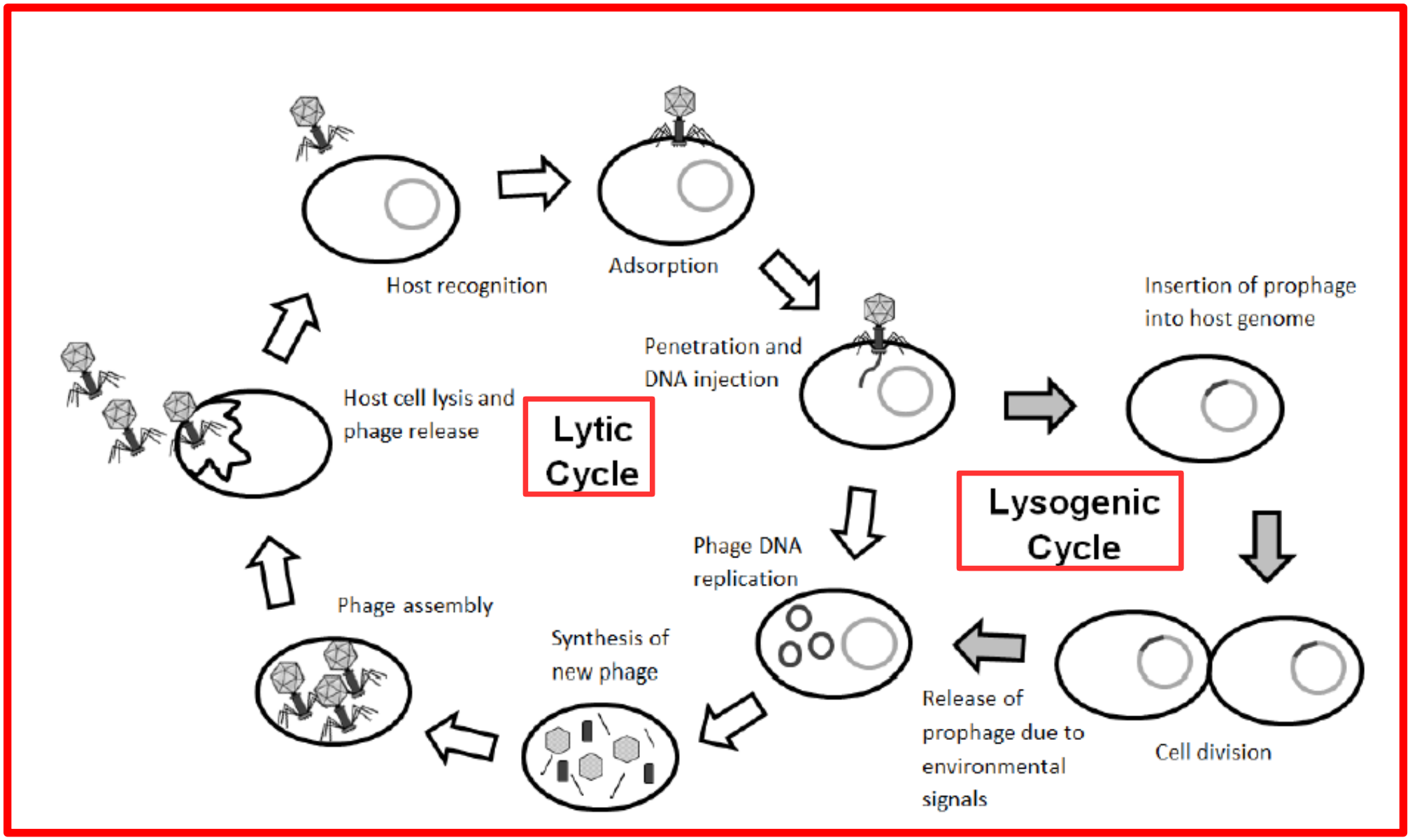
CYCLE LYTIQUE / CYCLE LYSOGENIQUE



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GEEPhage
Phagothérapie

OU TROUVE-T-ON DES PHAGES ?

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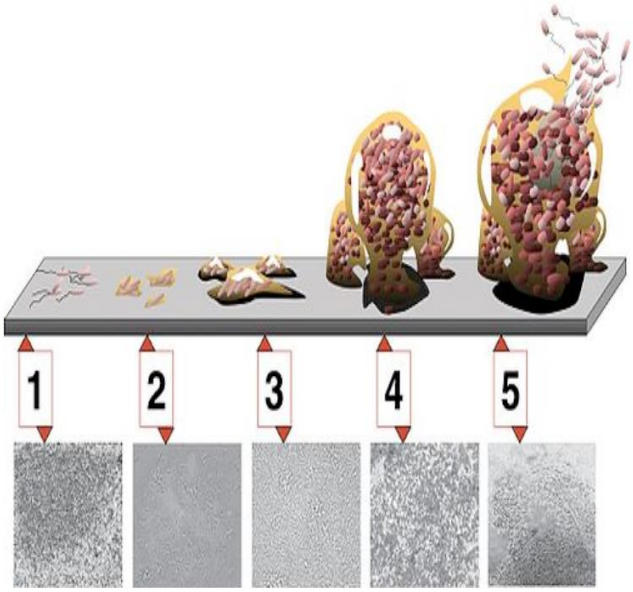
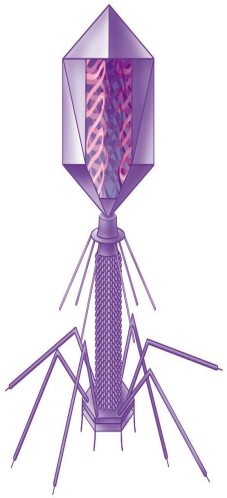
PHAGE ET BIOFILM

GEEPhage Phagothérapie





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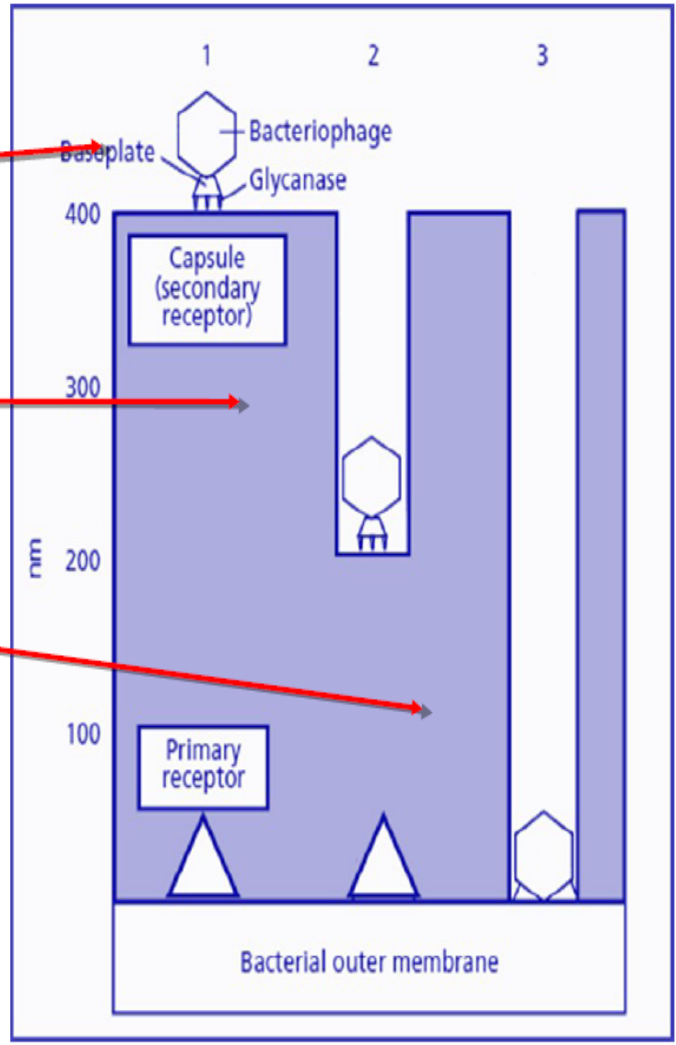

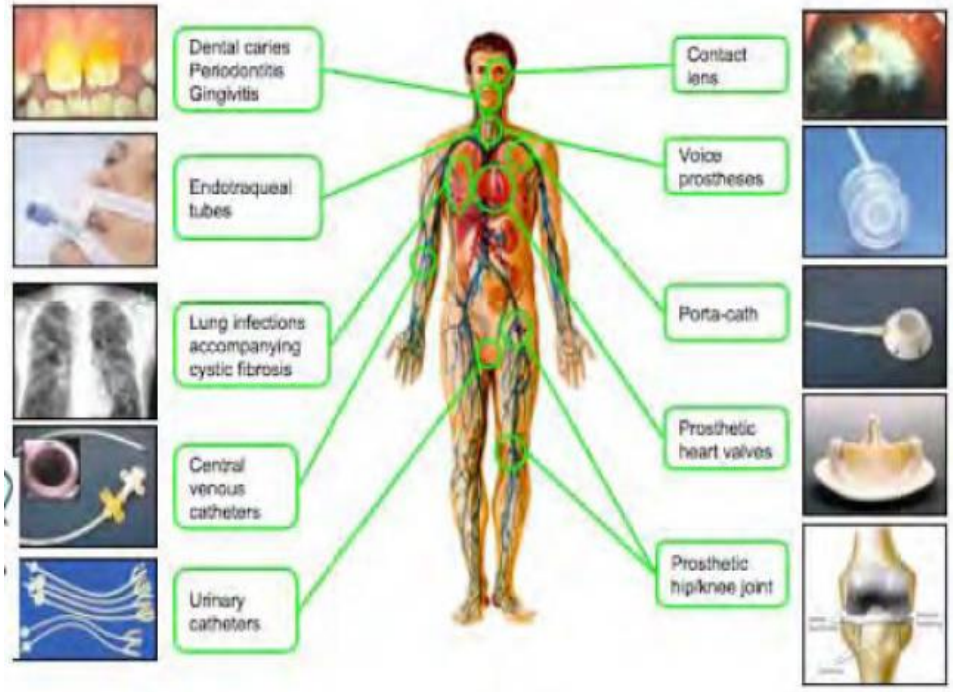

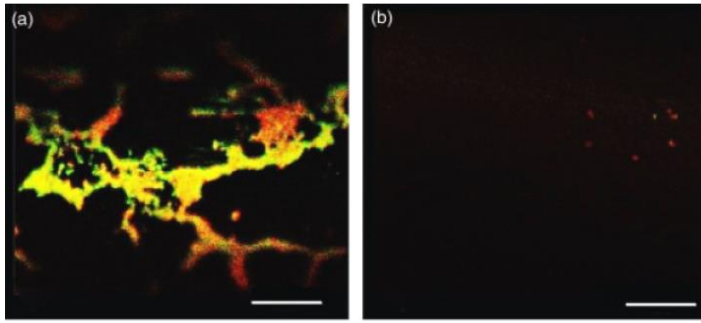

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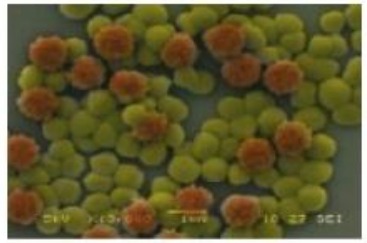
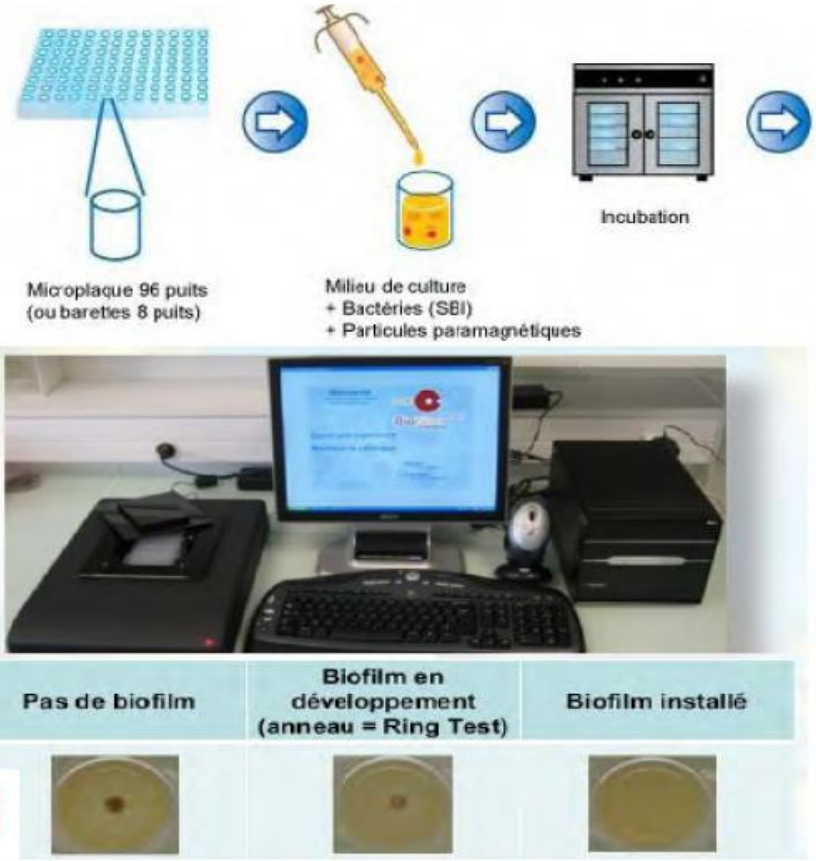
ANTIBIO-PHAGO-FILMOGRAMME

Modèles expérimentaux statiques

Biofilm Ring Test (BioFilm Control)

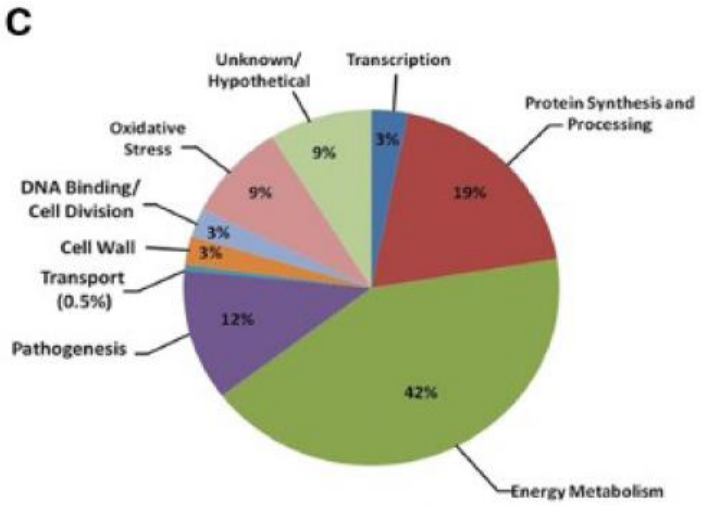




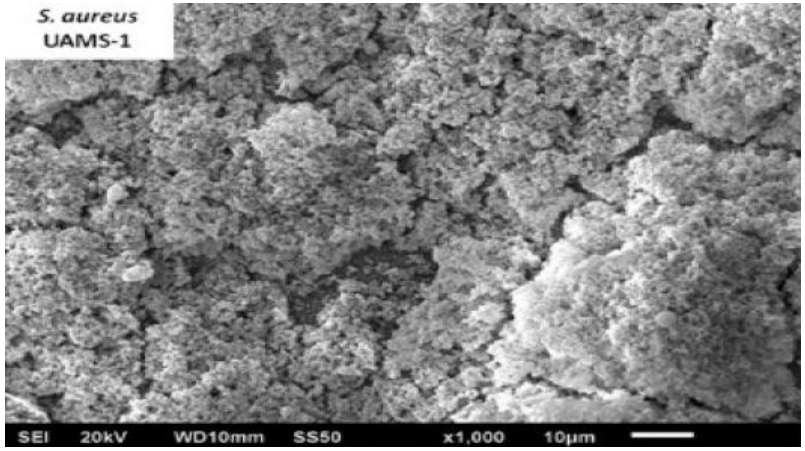
BioFilm Control

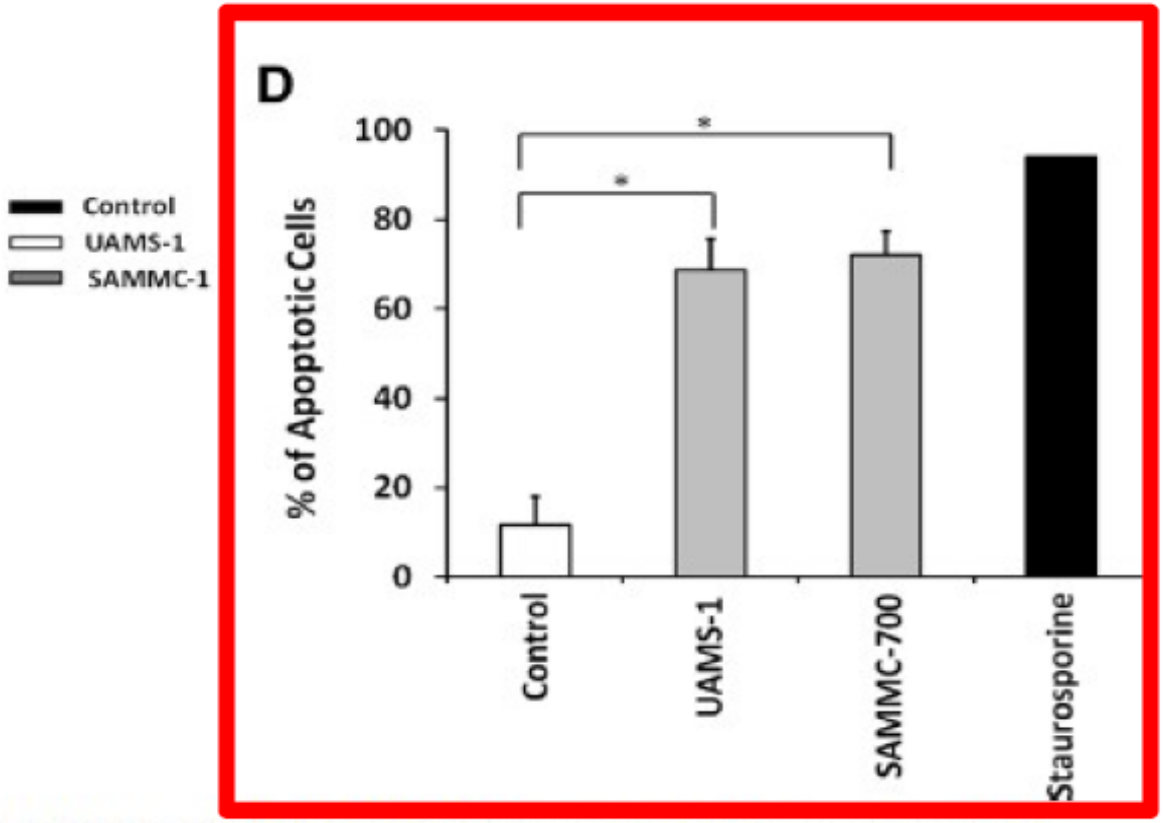
BIOFILM ET OSTEOBLASTOSE

Staphylococcus aureus biofilms decrease osteoblast viability, inhibits osteogenic differentiation, and increases bone resorption



S. aureus SAMCC 700 Biofilm Condition Media





Sanchez *et al. BMC Musculoskeletal Disorders* 2013, **14**:187
http://www.biomedcentral.com/1471-2474/14/187

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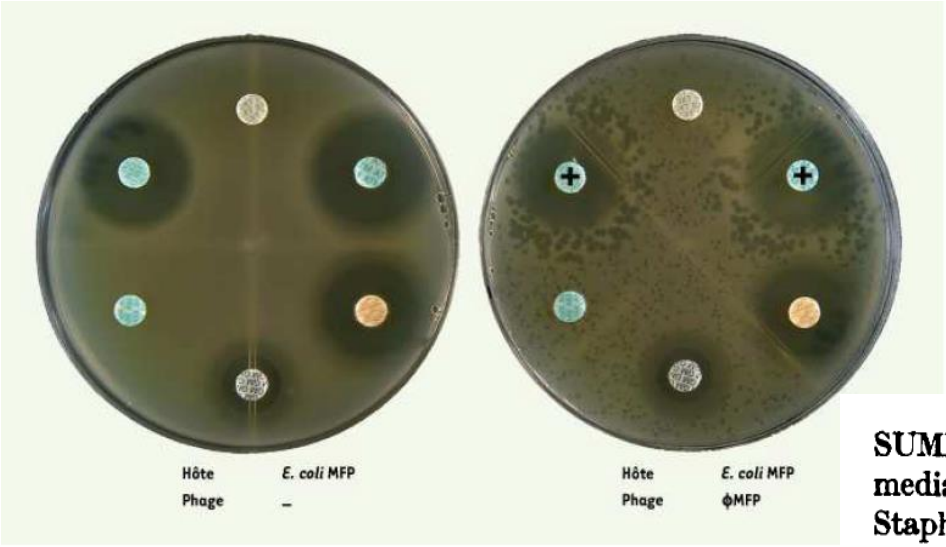
PHAGES ET ANTIBIOTIQUES

GEEPhage Phagothérapie

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



André M. Comeau[1*], Françoise Tétart[1*], Sabrina N. Trojet[1], Marie-Françoise Prère[1,2], H. M. Krisch[1*]

1 Laboratoire de Microbiologie et Génétique Moléculaires, Centre National de la Recherche Scientifique, Université Paul Sabatier-Toulouse, Toulouse, France, 2 Laboratoire de Bactériologie, Institut Fédératif de Biologie, Centre Hospitalier Universitaire de Toulouse, Toulouse, France

The Influence of Antibacterial Substances on the Interaction of Bacteria and Bacteriophages

1. The Influence of Penicillin

By W. J. ELFORD

National Institute for Medical Research, Hampstead, London

SUMMARY: Penicillin in concentrations up to 100 units/ml. in broth or synthetic media has no demonstrable effect, after 20 hr. incubation at 37°, on the activities of Staphylococcus K phage, Coli-phage C 36, Coli-dysentery phage S 13, a Streptococcal phage and a *Bacillus subtilis* phage.

The simultaneous action of penicillin and phage on young cultures of *Staphylococcus aureus* (Oxford) in broth or synthetic medium at 37° produces, under certain conditions, a more rapid lysis than occurs in the presence of penicillin or phage alone.

The phenomenon of accelerated lysis through the joint action of penicillin and phage occurs with other organisms besides *Staph. aureus*, e.g. *B. subtilis* and *Streptococcus pyogenes*, Group C, differing from that with *Staph. aureus* only in degree.

Penicillin does not affect the adsorption of phage by the organisms. When the amount of antibiotic is sufficient to interfere adversely with the growth of the cell then the multiplication of phage decreases. It is suggested that certain balanced intracellular reactions of metabolism are disturbed by the action of penicillin, and as a result, intermediates essential to growth both of cell and phage cease to be available.

A phage-inhibiting substance was demonstrable in certain instances when *Staph. aureus* (Oxford) cultures were lysed by penicillin.

Table 1. Examples of Studies Showing Antibiotic–Phage Positive Effects against Problematic or Model Bacteria

Bacteria	Phage Family	Antibiotic	Effects	Refs
Burkholderia cepacia	Myoviridae	Meropenem, ciprofloxacin, tetracycline	- PAS[a] - Increased survival of larvae	[40]
Escherichia coli	Not described	Enrofloxacin	- Total protection of birds	[17]
Escherichia coli	Myoviridae	Cefotaxime	- PAS - Eradication of bacterial biofilms	[37]
Klebsiella pneumoniae	Podoviridae (T7-like)	Ciprofloxacin	- Eradication of bacteria - Prevention of resistant variants	[27]
Pseudomonas aeruginosa	Podoviridae	Streptomycin	- Decreased bacterial density - Limited antibiotic resistance	[32]
Pseudomonas aeruginosa	Siphoviridae	Ceftriaxone	- Synergistic reduction of bacterial growth	[41]
Pseudomonas fluorescens	Podoviridae	Kanamycin	- Decreased bacterial survival - Limited antibiotic resistance	[29]
Staphylococcus aureus	Myoviridae	Linezolid	- Stopped MRSA hindpaw foot infection - Decreased bacterial density	[28]
Staphylococcus aureus	Myoviridae	Gentamicin	- Decreased bacterial density - Prevention of phage-resistant variants	[30]

[a]Phage–antibiotic synergy.

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

Sanjay Chhibber*, Tarsem Kaur, Sandeep Kaur
Department of Microbiology, Panjab University, Chandigarh, India

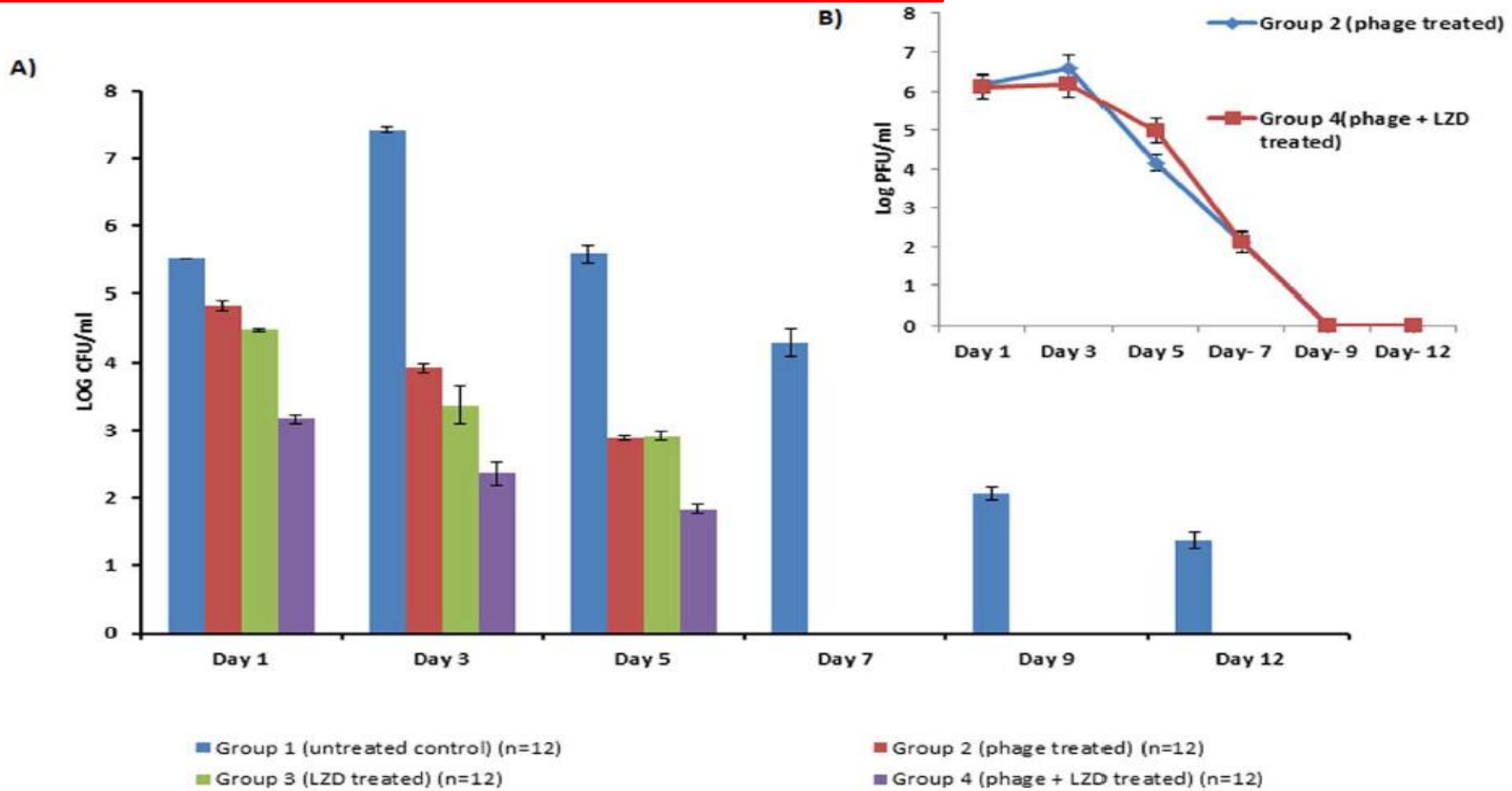

Figure 4. Bacterial load (in terms of Log CFU/ml) in A) Hindpaws of diabetic BALB/c mice following treatment with phage MR-10, linezolid and combination of phageMR-10 and linezolid (25 mg/kg/per oral) and Phage titers(in terms of Log PFU/ml) in B) Hindpaws of phage treated (group 2) and phage + LZD treated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

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The Journal of Infectious Diseases

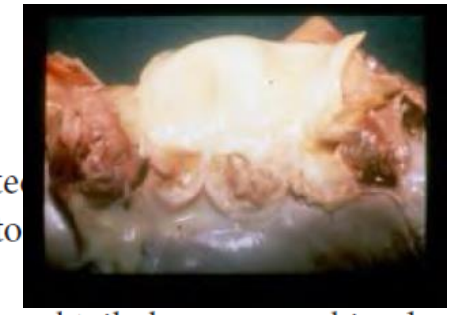
MAJOR ARTICLE

IDSA Infectious Diseases Society of America
hivma hiv medicine association
OXFORD

Synergistic Interaction Between Phage Therapy and Antibiotics Clears *Pseudomonas Aeruginosa* Infection in Endocarditis and Reduces Virulence

Frank Oechslin,[1] Philippe Piccardi,[1] Stefano Mancini,[1] Jérôme Gabard,[3] Philippe Moreillon,[1] José M. Entenza,[1] Gregory Resch,[1] and Yok-Ai Que[2]

[1]Department of Fundamental Microbiology, University of Lausanne, and [2]Department of Intensive Care Medicine, Bern University Hospital, Switzerland; and [3]Pherecydes Pharma, Romainville, France



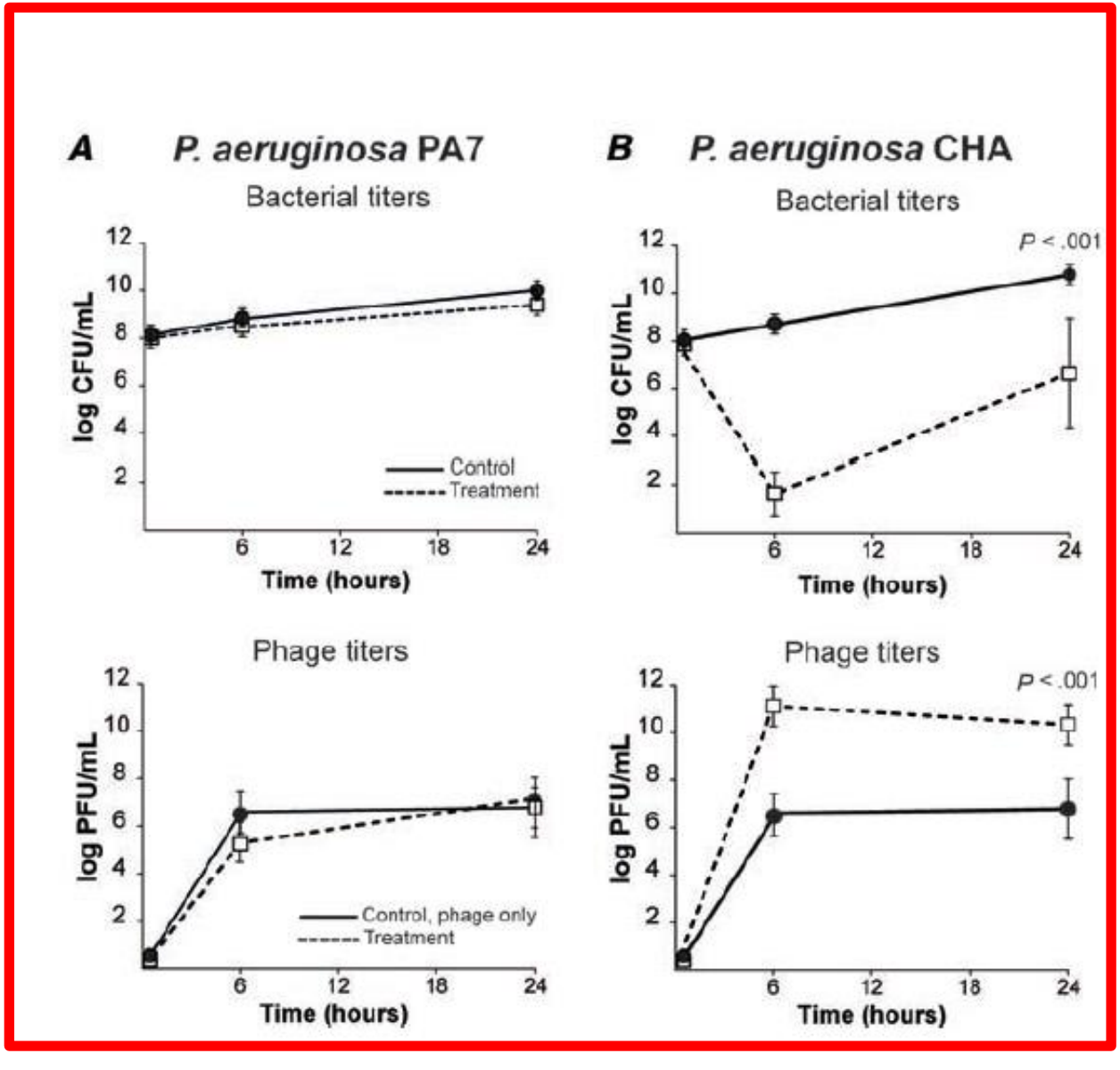
Background. Increasing antibiotic resistance warrants therapeutic alternatives. Here we investigate phage-therapy (phage) alone or combined with antibiotics against experimental endocarditis (EE) due to an archetype of difficult-to-treat infection.

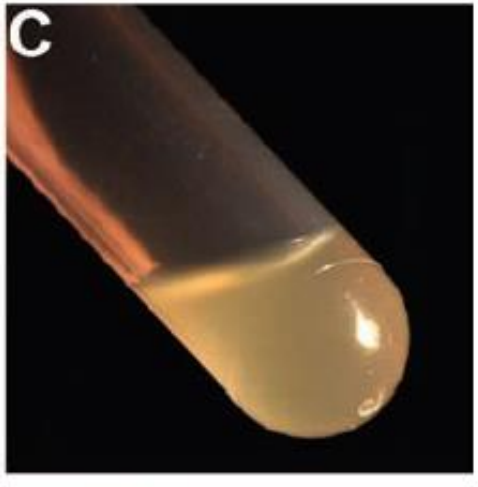
Methods. In vitro fibrin clots and rats with aortic EE were treated with an antipseudomonas phage cocktail alone or combined with ciprofloxacin. Phage pharmacology, therapeutic efficacy, and resistance were determined.

Results. In vitro, single-dose phage therapy killed 7 log colony-forming units (CFUs)/g of fibrin clots in 6 hours. Phage-resistant mutants regrew after 24 hours but were prevented by combination with ciprofloxacin (2.5 × minimum inhibitory concentration). In vivo, single-dose phage therapy killed 2.5 log CFUs/g of vegetations in 6 hours ($P < .001$ vs untreated controls) and was comparable with ciprofloxacin monotherapy. Moreover, phage/ciprofloxacin combinations were highly synergistic, killing >6 log CFUs/g of vegetations in 6 hours and successfully treating 64% (n = 7/11) of rats. Phage-resistant mutants emerged in vitro but not in vivo, most likely because resistant mutations affected bacterial surface determinants important for infectivity (eg, the *pilT* and *galU* genes involved in pilus motility and LPS formation).

Conclusions. Single-dose phage therapy was active against *P. aeruginosa* EE and highly synergistic with ciprofloxacin. Phage-resistant mutants had impaired infectivity. Phage-therapy alone or combined with antibiotics merits further clinical consideration.

Keywords. bacteriophage; phage therapy; endocarditis; *Pseudomonas aeruginosa*; resistance; antibiotic.





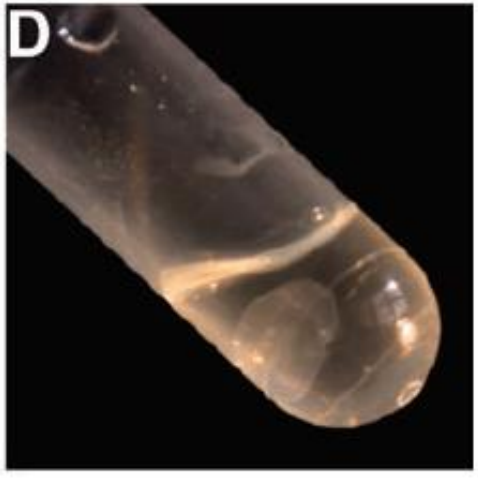


Figure 1. Activity of phage cocktail PP1131 in in vitro fibrin clots. Clots were produced from rat plasma and infected with 10^8 colony-forming units (CFUs)/mL of either phage-resistant *Pseudomonas aeruginosa* strain PA7 (*A*) or phage-susceptible strain CHA (*B*). Clots were left untreated (solid lines) or exposed to 10^8 PFUs/mL of PP1131 for 24 hours at 37°C (dashed lines). Phage titers in noninfected clots (solid lines) and in PA7-infected or CHA-infected clots (dashed lines) were determined 6 hours and 24 hours

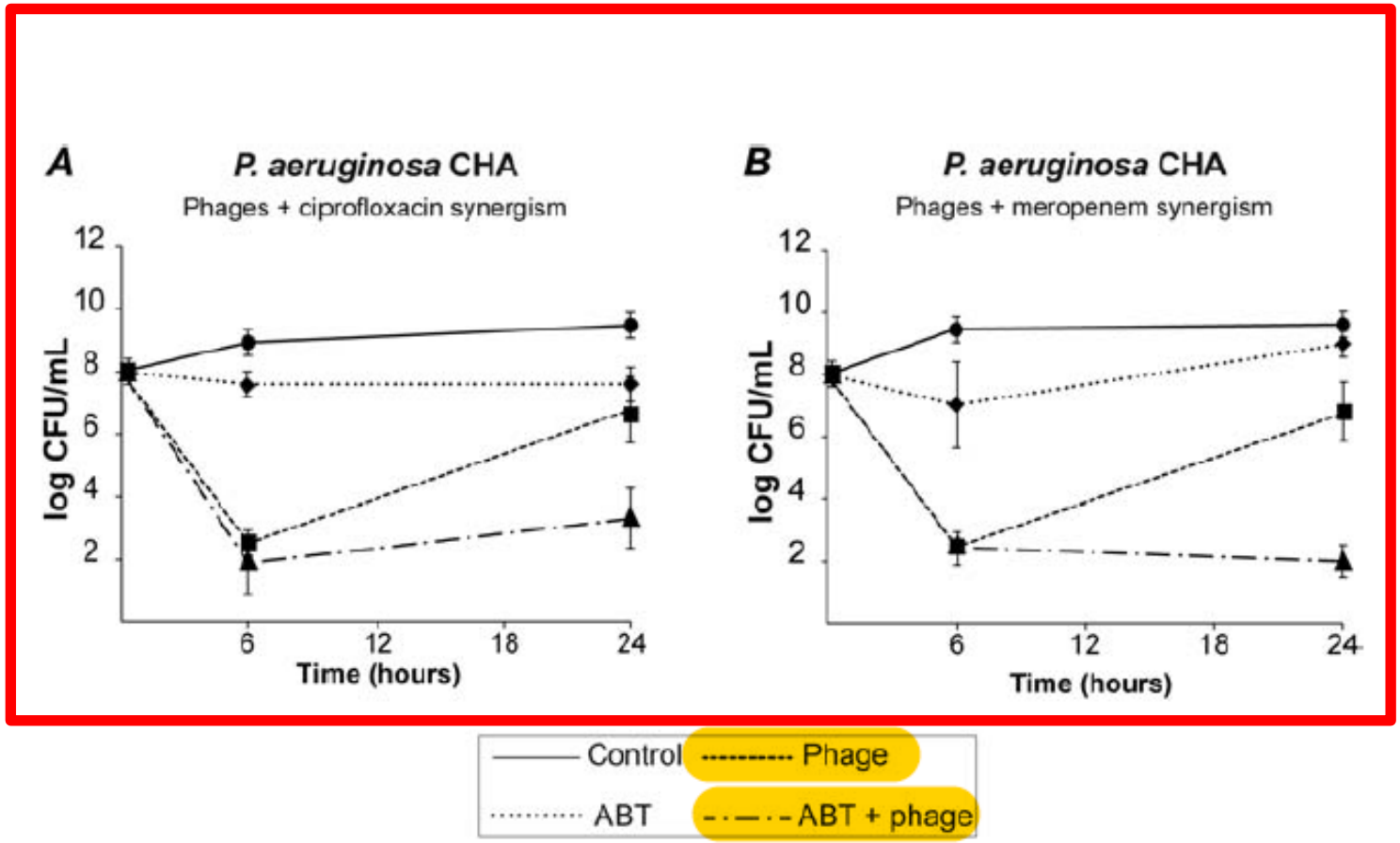


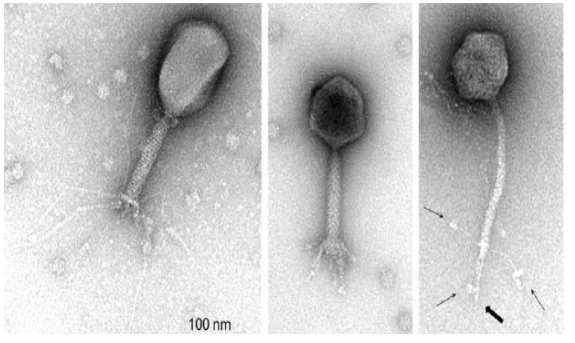
Figure 2. Bactericidal synergism between phages and selected antibiotics. Bacterial killing by phage-antibiotic combinations was tested by using 10^8 PFUs/mL of PP1131 with 2.5-times the minimum inhibitory concentration (MIC) of ciprofloxacin (*A*) or meropenem (*B*) (MIC of 0.19 μg/mL and 0.125 μg/mL, respectively). Each value represents the mean ± SD of 4–16 independent clots. Abbreviation: ABT, antibiotic.

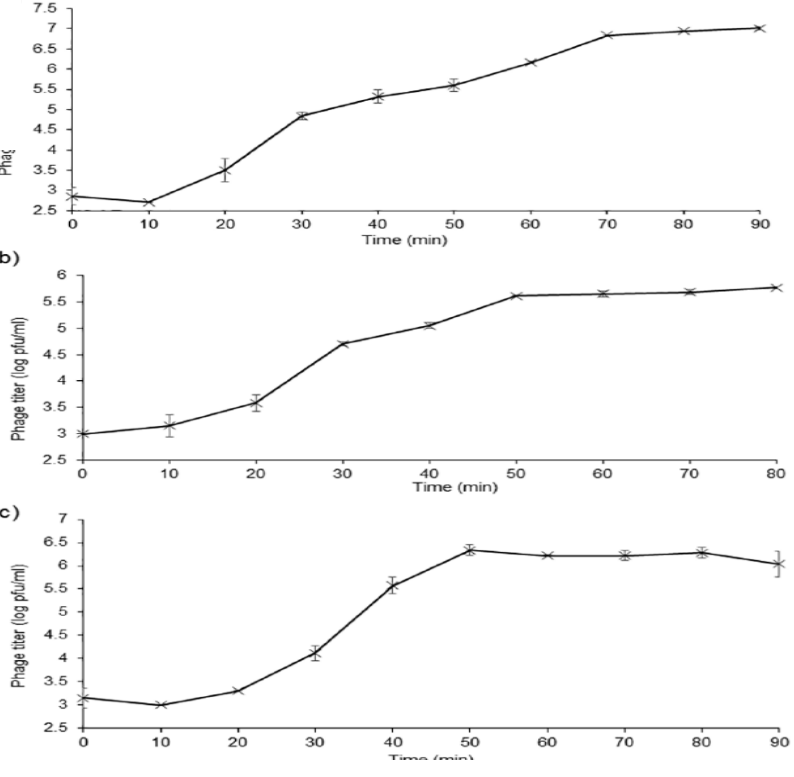
Three New *Escherichia coli* Phages from the Human Gut Show Promising Potential for Phage Therapy

Marion Dalmasso[1,2¤], Ronan Strain[1,2], Horst Neve[3], Charles M. A. P. Franz[3], Fabien J. Cousin[1,2¤], R. Paul Ross[2,4], Colin Hill[1,2]*

1 School of Microbiology, University College Cork, Cork, Ireland, 2 APC Microbiome Institute, University

With the emergence of multi-drug resistant bacteria the use of bacteriophages (phages) is gaining renewed interest as promising anti-microbial agents. The aim of this study was to isolate and characterize phages from human fecal samples. Three new coliphages, ϕAPCEc01, ϕAPCEc02 and ϕAPCEc03, were isolated. Their phenotypic and genomic characteristics, and lytic activity against biofilm, and in combination with ciprofloxacin, were investigated. All three phages reduced the growth of *E. coli* strain DPC6051 at multiplicity of infection (MOI) between 10^{-3} and 10^{5}. A cocktail of all three phages completely inhibited the growth of *E. coli*. The phage cocktail also reduced biofilm formation and prevented the emergence of phage-resistant mutants which occurred with single phage. When combined with ciprofloxacin, phage alone or in cocktail inhibited the growth of *E. coli* and prevented the emergence of resistant mutants. These three new phages are promising biocontrol agents for *E. coli* infections.





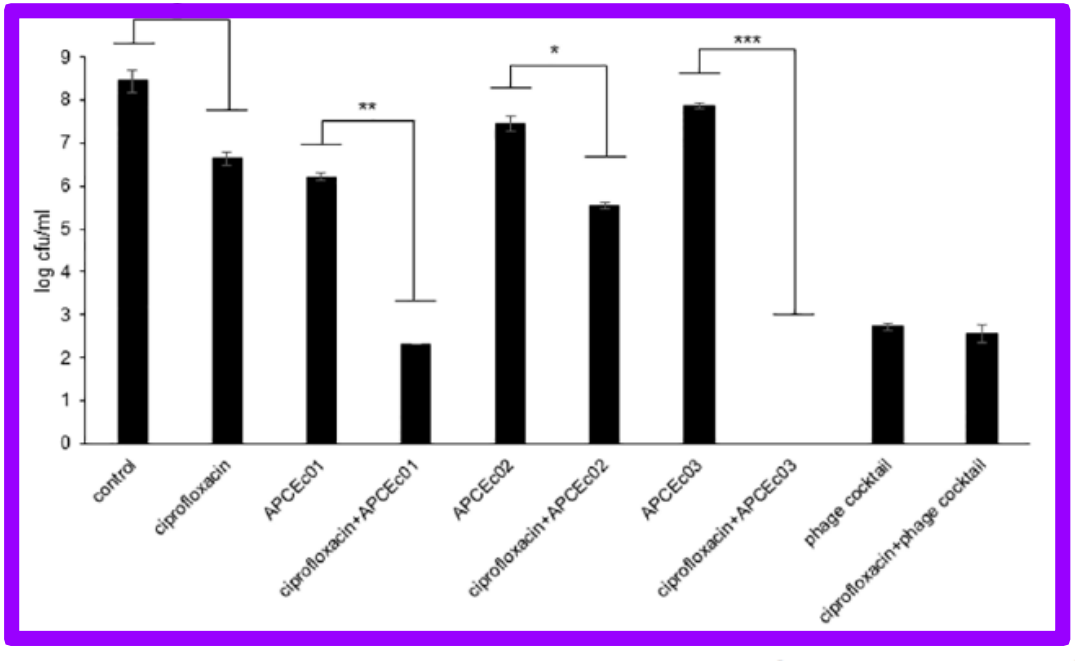


Fig 6. Effect of a combination of ciprofloxacin HCl and phages ϕAPCEc01, ϕAPCEc02, and ϕAPCEc03, alone or in cocktail, on the growth of *E. coli* strain DPC6051. Each condition was tested in

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Phagothérapie

Bacteriophages as Potential Treatment for Urinary Tract Infections

*Wilbert Sybesma[1], Reinhard Zbinden[2], Nino Chanishvili[3], Mzia Kutateladze[3], Archil Chkhotua[4], Aleksandre Ujmajuridze[4], Ulrich Mehnert[1] and Thomas M. Kessler[1]**

[1] Neuro-Urology, Spinal Cord Injury Center and Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland, [2] Institute of Medical Microbiology, University of Zürich, Zürich, Switzerland, [3] The Eliava Institute of Bacteriophage, Microbiology, and Virology, Tbilisi, Georgia, [4] Tsulukidze National Center of Urology, Tbilisi, Georgia

Background: Urinary tract infections (UTIs) are among the most prevalent microbial diseases and their financial burden on society is substantial. The continuing increase of antibiotic resistance worldwide is alarming so that well-tolerated, highly effective therapeutic alternatives are urgently needed.

Objective: To investigate the effect of bacteriophages on *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from the urine of patients suffering from UTIs.

Material and methods: Forty-one *E. coli* and 9 *K. pneumoniae* strains, isolated from the urine of patients suffering from UTIs, were tested *in vitro* for their susceptibility toward bacteriophages. The bacteriophages originated from either commercially available bacteriophage cocktails registered in Georgia or from the bacteriophage collection of the George Eliava Institute of Bacteriophage, Microbiology and Virology. *In vitro* screening of bacterial strains was performed by use of the spot-test method. The experiments were implemented three times by different groups of scientists.

Results: The lytic activity of the commercial bacteriophage cocktails on the 41 *E. coli* strains varied between 66% (Pyo bacteriophage) and 93% (Enko bacteriophage). After bacteriophage adaptation of the Pyo bacteriophage cocktail, its lytic activity was increased from 66 to 93% and only one *E. coli* strain remained resistant. One bacteriophage of the Eliava collection could lyse all 9 *K. pneumoniae* strains.

Conclusions: Based on the high lytic activity and the potential of resistance optimization by direct adaption of bacteriophages as reported in this study, and in view of the continuing increase of antibiotic resistance worldwide, bacteriophage therapy is a promising treatment option for UTIs highly warranting randomized controlled trials.

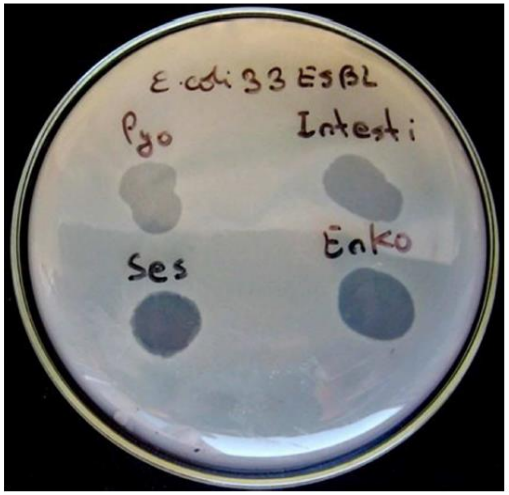

FIGURE 1 | Plaque morphology of *Escherichia coli* strain #33. The figure shows overgrown (partial) lysis (OL) in case of Pyo and Intesti bacteriophages and confluent (complete) lysis (CL) in case of Ses and Enko bacteriophages. All these results are positive.

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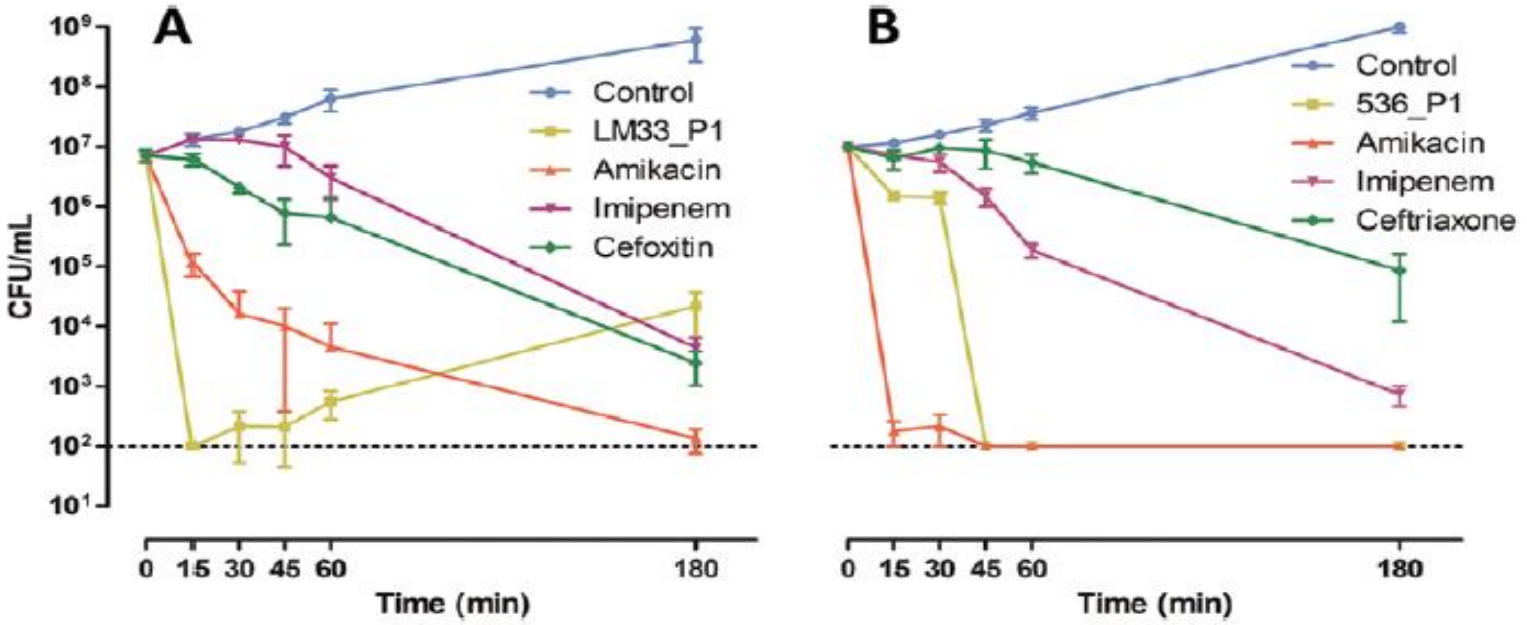
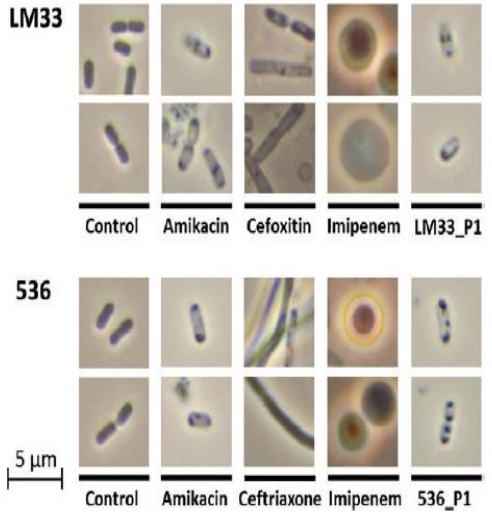
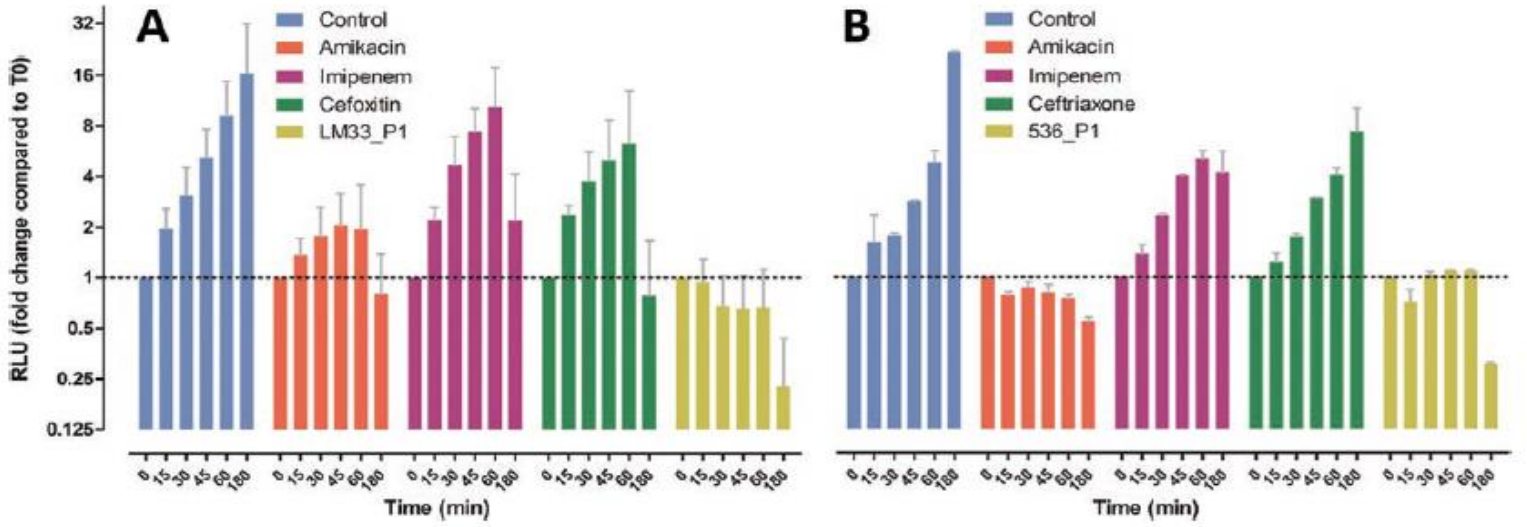
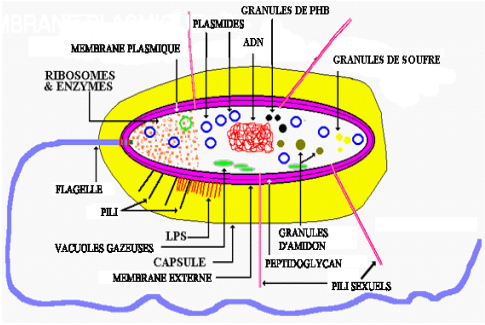
The Lysis of Pathogenic *Escherichia coli* by Bacteriophages Releases Less Endotoxin Than by β-Lactams

Nicolas Dufour,[1,2,3] Raphaëlle Delattre,[1,3,4] Jean-Damien Ricard,[2,3,5] and Laurent Debarbieux[1]









Clinical Infectious Diseases

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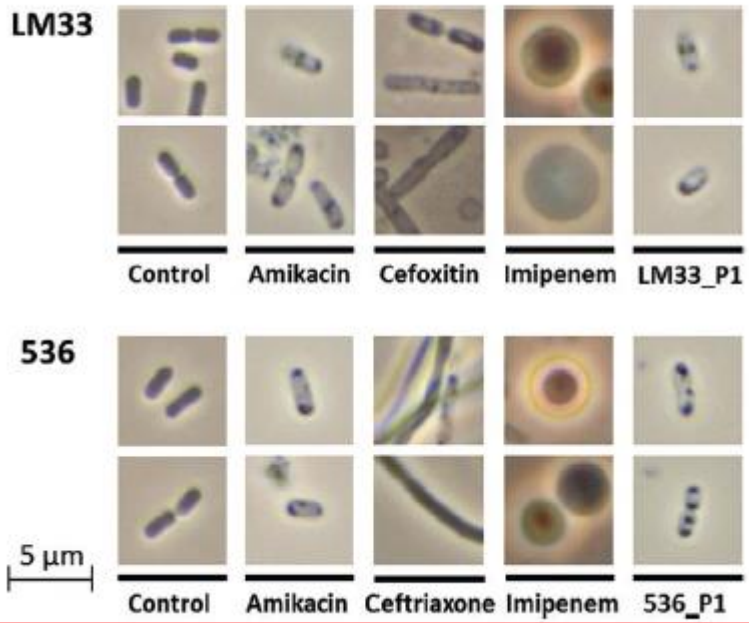

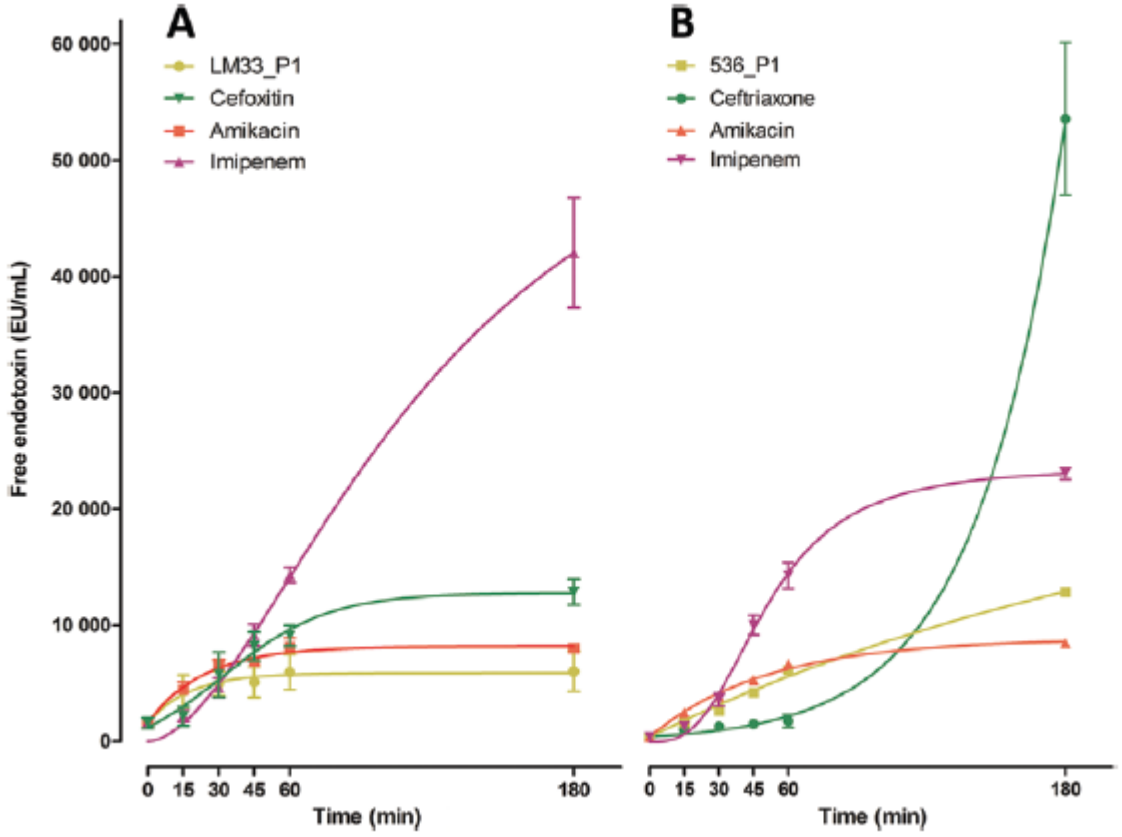

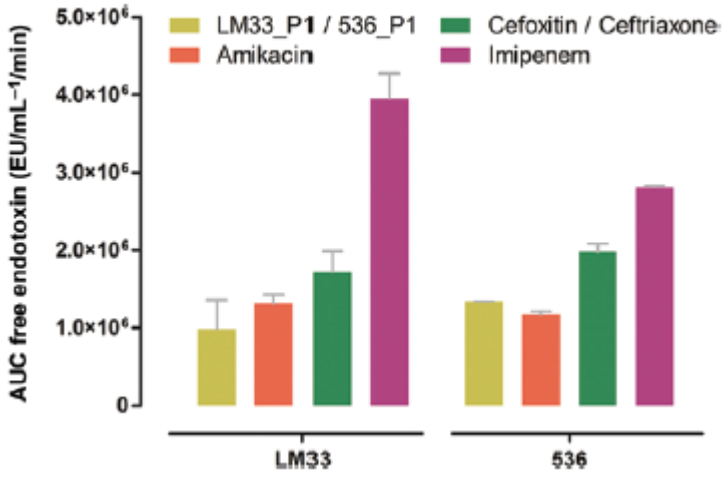

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Phages and immunomodulation

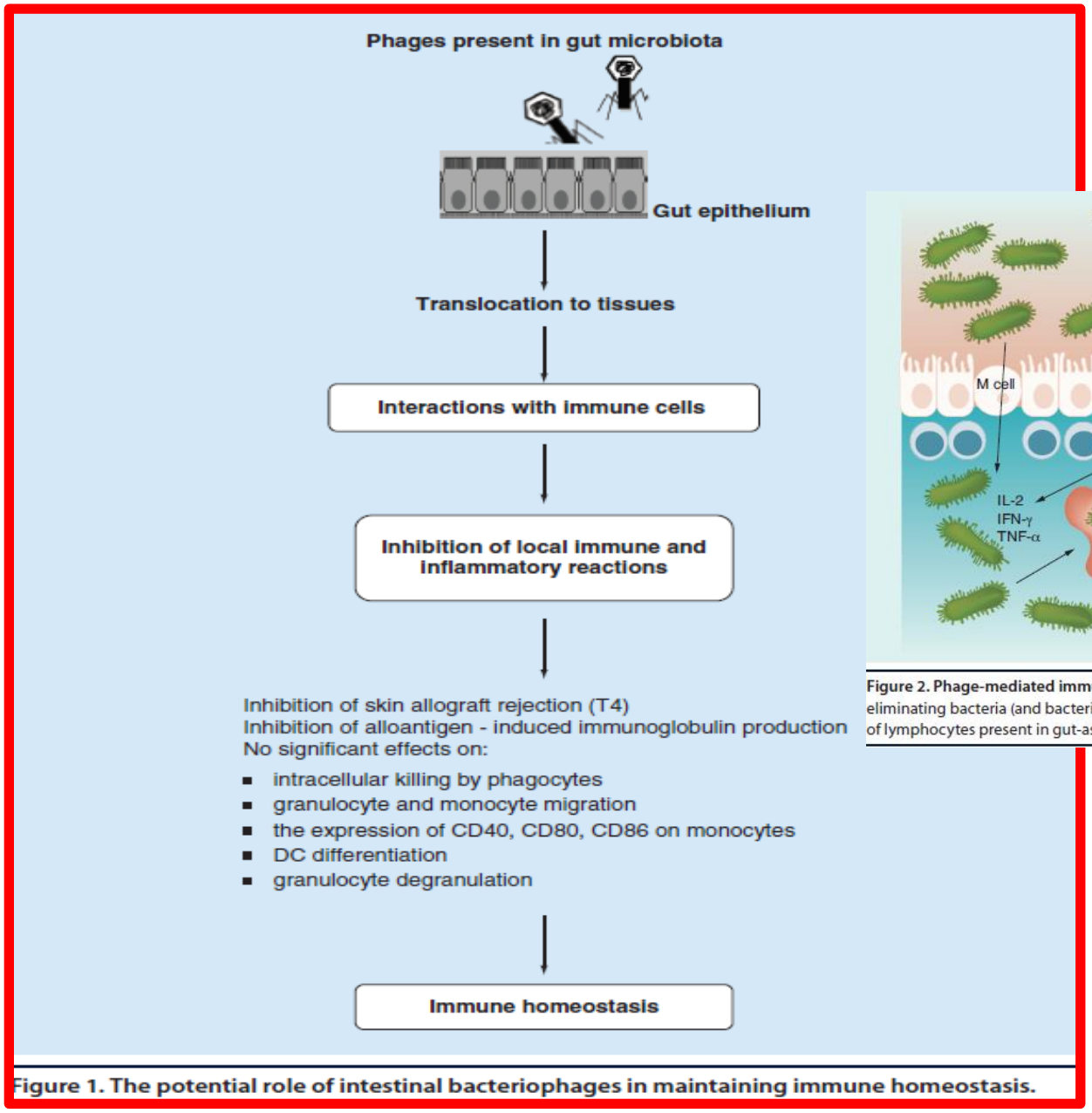


Figure 1. The potential role of intestinal bacteriophages in maintaining immune homeostasis.

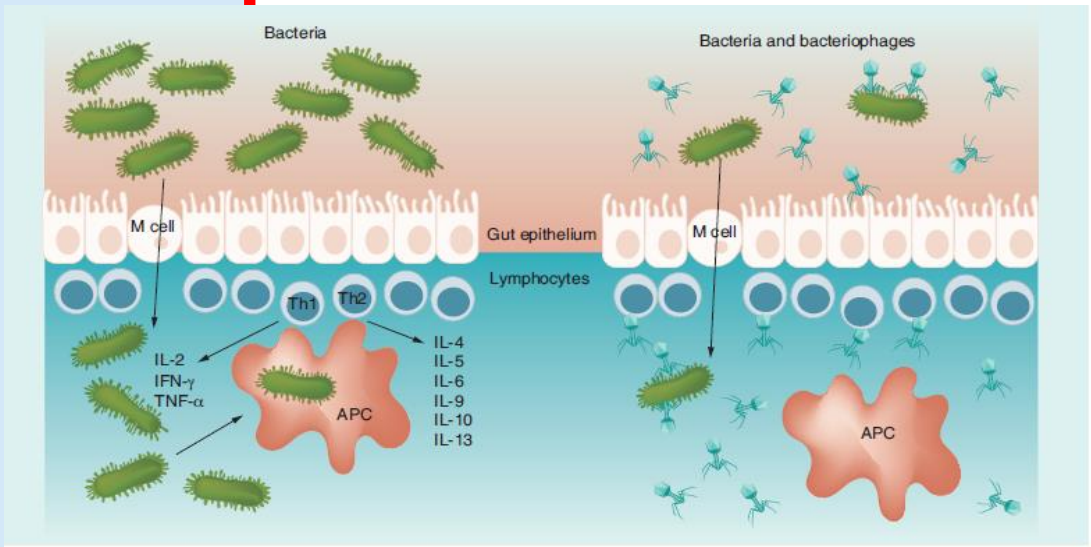


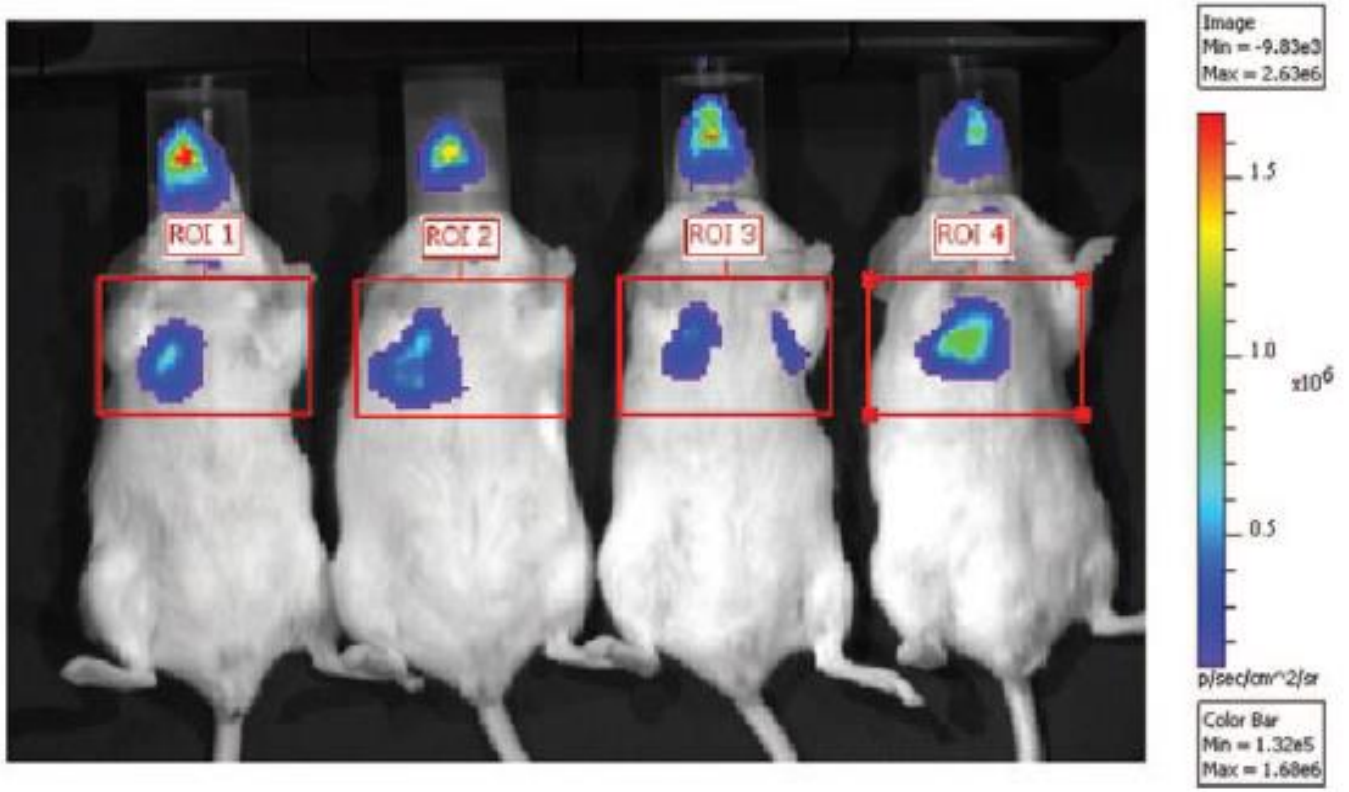
Figure 2. Phage-mediated immunoregulation. Phages can downregulate both Th1- and Th2-derived cytokine production by eliminating bacteria (and bacteria-dependent upregulation of immunity and inflammation). They can also directly control the activity of lymphocytes present in gut-associated lymphoid tissue. Phages that translocate can also act at different tissues (**Figure 1**).

10.2217/fmb-2017-0049 © Andrzej Górski

Future Microbiol. (Epub ahead of print)

Andrzej Górski[*,1,2,3], Krystyna Dąbrowska[1], Ryszard Międzybrodzki[1,2,3], Beata Weber-Dąbrowska[1,2], Marzanna Łusiak-Szelachowska[1], Ewa Jończyk-Matysiak[1] & Jan Borysowski[3]

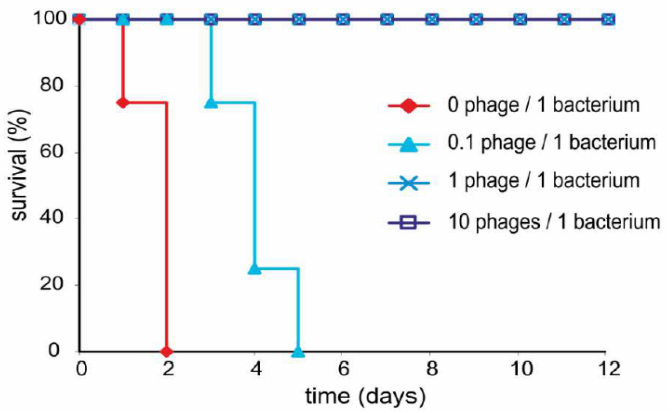
LES MODELES ANIMAUX

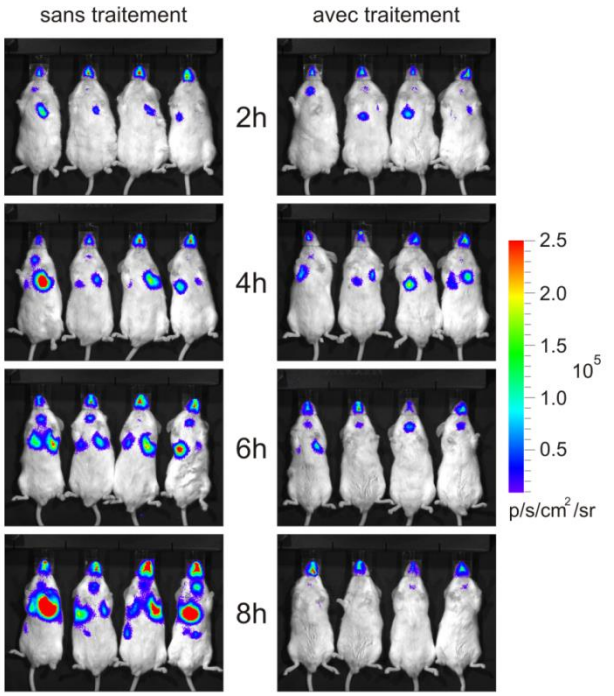


Infection pulmonaire aiguë à *P. aeruginosa*

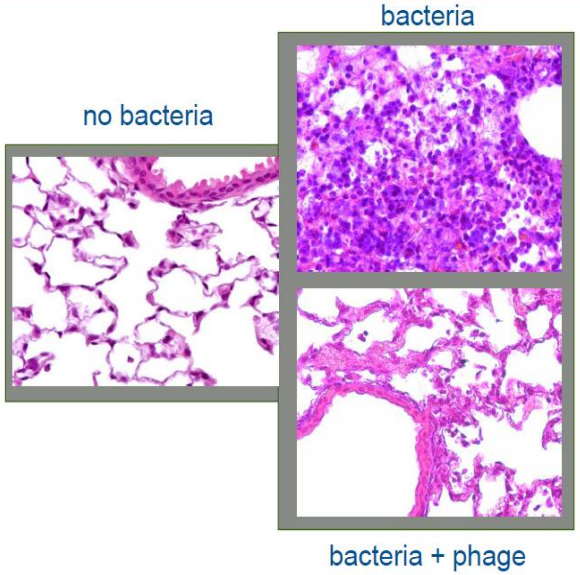
Bacteriophages treatment of lung infection in mice

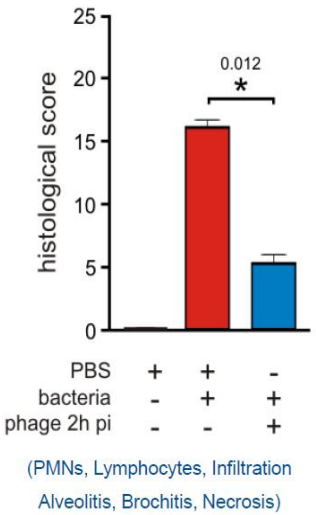
Infection by $1.0x10^7$ bacteria and 2H later different doses of phages





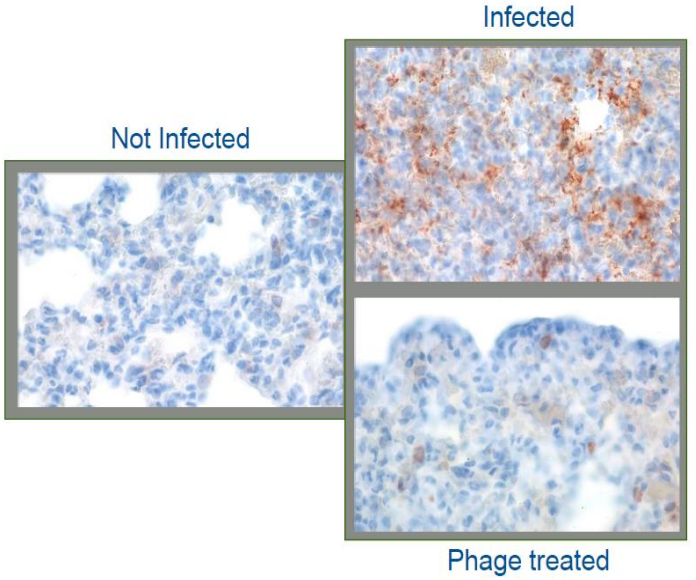
Histology on bacteriophage-treated lungs





(PMNs, Lymphocytes, Infiltration
Alveolitis, Brochitis, Necrosis)

Immuno-histochemistry on bacteriophage-treated lungs



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Treatment of Highly Virulent Extraintestinal Pathogenic *Escherichia coli* Pneumonia With Bacteriophages*

Nicolas Dufour, MD, MSc[1,2,3,4]; Laurent Debarbieux, PhD[1]; Mélanie Fromentin, MSc[2]; Jean-Damien Ricard, MD, PhD[2,3,4]

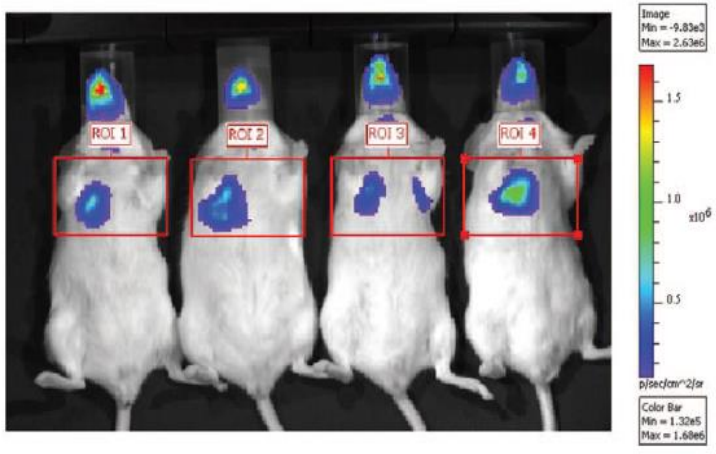
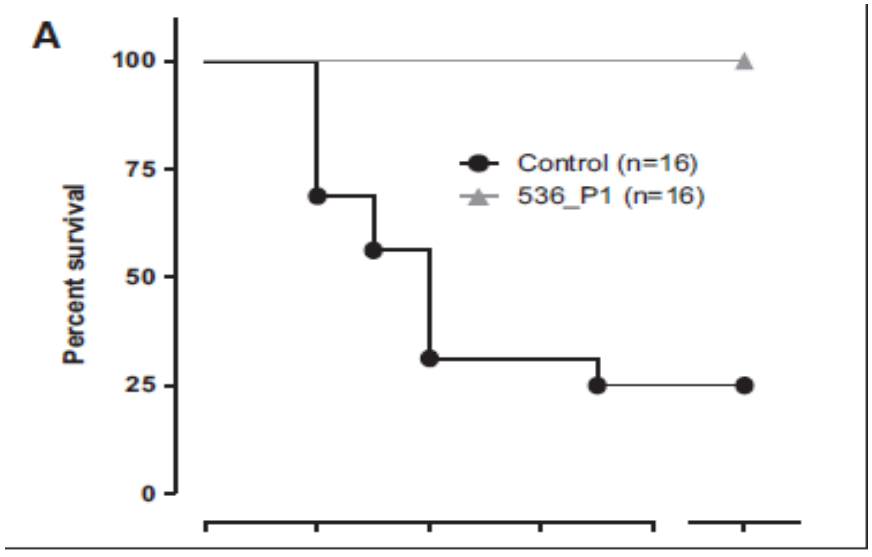
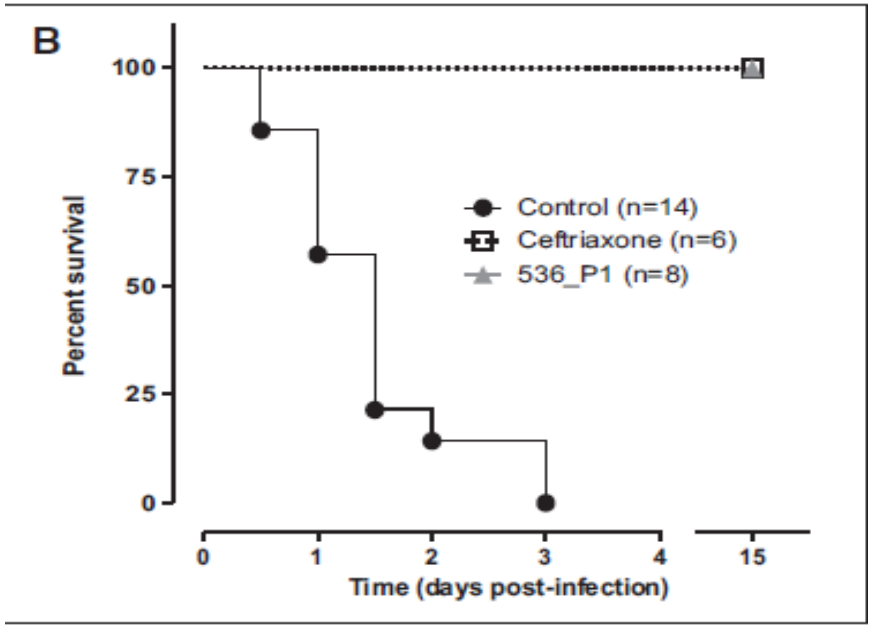


TABLE 1. Bacterial and Bacteriophage Counts on Lung Homogenates and Data Obtained From Bronchoalveolar Lavage Fluids

Variable	Uninfected Animals	2 Hr Post Infection (Without Treatment)	6 Hr Post Infection		16 Hr Post Infection	
			PBS (Control)	536_P1 (Treatment)	PBS (Control)	536_P1 (Treatment)
Bacterial count (lung homogenate, CFU/g)	NA	1.0×10^8 [4.5×10^7; 1.6×10^8]	8.3×10^6 [5.2×10^6; 1.0×10^7]	4.3×10^5 [2.0×10^5; 1.3×10^6][a]	4.8×10^8 [2.1×10^8; 5.5×10^8]	2.4×10^6 [2.1×10^5; 1.8×10^7][a]
Bacteriophage count (lung homogenate, PFU/g)	NA	NA	0	8.8×10^8 [2.6×10^8; 1.4×10^9]	0	1.1×10^{10} [2.5×10^9; 1.5×10^{10}][b]
Bronchoalveolar lavage fluid analysis						
Total nucleated cell count (cells/mL)	$1.3\times10^6\pm2.3\times10^5$	NA	3.1×10^6 ($\pm9.7\times10^5$)	3.9×10^6 ($\pm9.4\times10^5$)	1.0×10^7 ($\pm2.3\times10^6$)	1.0×10^7 ($\pm4.5\times10^6$)[b]
Ratio of polymorphonuclears/ monocyte-macrophages (%)	4.2/95.8 (± 1.5)	NA	93.2/6.8 (± 2.7)	92.2/7.8 (± 4.2)	96.8/3.2 (± 1.8)	91.8/8.2 (± 4.8)[a]
Percentage of phagocytes with engulfed bacteria (%)	NA	NA	NA	NA	26.6 (± 6.8)	0.6 (±0.2)[a]
Total protein (µg/mL)	74 (± 6)	NA	140 (± 59)	208 (± 44)	320 (± 62)	304 (± 76)[b]
LDH activity (fold change compared with noninfected condition)	1	NA	1.3 (± 0.3)	1.3 (± 0.2)	3.9 (± 0.7)	3.1 (± 0.6)[b]
KC/CXCL-1 (pg/mL)	24 (± 12)	NA	14,816 (± 3,720)	17,729 (± 2,531)	14,927 (± 1,044)	3,487 (± 1,264)[a]





Phage therapy of staphylococcal chronic osteomyelitis in experimental animal model

Chandan Kishor[1,†], Raghvendra Raman Mishra[2,†], Shyam K. Saraf[1], Mohan Kumar[3], Arvind K. Srivastav[4] & Gopal Nath[2]

Background & objectives: Methicillin resistant *Staphylococcus aureus* (MRSA) are the commonest cause of osteomyelitis. The aim of this study was to evaluate the role of an alternative therapy *i.e.* application of *S. aureus* specific bacteriophages in cases of osteomyelitis caused by MRSA in animal model.

Methods: Twenty two rabbits were included in this study. The first two rabbits were used to test the safety of phage cocktail while the remaining 20 rabbits were divided into three groups; group A (n=4) to assess the establishment of osteomyelitis; group B (n=4) osteomyelitis developed but therapy started only after six weeks; and group C (n=12) osteomyelitis developed and therapy started after three weeks. Groups B and C rabbits were treated with four doses of cocktail of seven virulent bacteriophages at the interval of 48 h. Comparison between three groups was made on the basis of observation of clinical, radiological, microbiological, and histopathological examinations.

Results: Experimental group rabbits recovered from the illness in the subsequent two weeks of the therapy. Appetite and activity of the rabbits improved, local oedema, erythema and induration subsided. There were minimal changes associated with osteomyelitis in X-ray and histopathology also showed no signs of infection with new bone formation. Control B group rabbits also recovered well from the infection.

Interpretation & conclusions: The present study shows a potential of phage therapy to treat difficult infections caused by multidrug resistant bacteria.

Table. Bacteriophage therapy of acute and chronic osteomyelitis in rabbit model

Group A	3rd wk	4th wk	5th wk	6th wk	7th wk onwards
1	C1,R1	C1,R2,H1	Two rabbits scarified on day 5 in 4th week		
2	C1,R2	C1,R2,H1			
3	C1,R2	C1,R2,	C1,R3	C1,R3,H1	Two rabbits sacrificed on day 5 in 6th week
4	C1,R2	C1,R2	C1,R2	C1,R3,H1	
Group C	Phage given to all the 12 rabbits on days 1,3,5,7 of 3rd week				
1	C1,R2,H1	This rabbit sacrificed on day 5 in 3th week			
2	C1,R2	C2,R2,H2	Three rabbits sacrificed on day 5 in 4th week		
3	C1,R1	C2,R2,H2			
4	C1,R2	C2,R2,H2			
5	C1,R1	C2,R2	C2,R5,H3	Four rabbits sacrificed on day 5 in 5th wk	
6	C1,R2	C2,R2	C2,R5,H3		
7	C1,R2	C2,R2	C2,R5,H3		
8	C1,R2	C2,R2	C2,R2,H2		
9	C1,R2	C2,R2	C2,R5	C2,R5,H3	Four rabbits sacrificed on day 5 in 6th week
10	C1,R1	C2,R2	C2,R5	C2,R5,H3	
11	C1,R2	C2,R2	C2,R2	C2,R5,H3	
12	C1,R2	C2,R2	C2,R2	C2,R2,H3	
Group B					Phage therapy given on days 1,3,5,7 after 6th week. These rabbits were not sacrificed and observed for 8 weeks**
1	C1,R1	C1,R2	C1,R3	C1,R3	C2R3
2	C1,R1	C1,R2	C1,R3	C1,R3	C2R3
3	C1,R2	C1,R3	C1,R3	C1,R4	C2R4
4	C1,R2	C1,R2	C1,R3	C1,R3	C2R3

Culture: C1, positive culture; C2, negative culture; Radiograph: R1-cortical erosion; R2, cortical erosion, sclerosis, osteolysis; R3, cortical errosion, osteolysis, sclerosis and sequestrum formation; R4, cortical errosion, osteolysis, sequestrum formation and arthritis of the knee; R5, new bone formation with decreased osteolysis. Histopathology: H1, marked inflammation and necrosis; H2, minimal inflammation and necrosis; H3, no inflammation, minimal necrosis and new bone formation.
All four rabbits of group B were kept for long term observation to see changes in clinical and radiological features

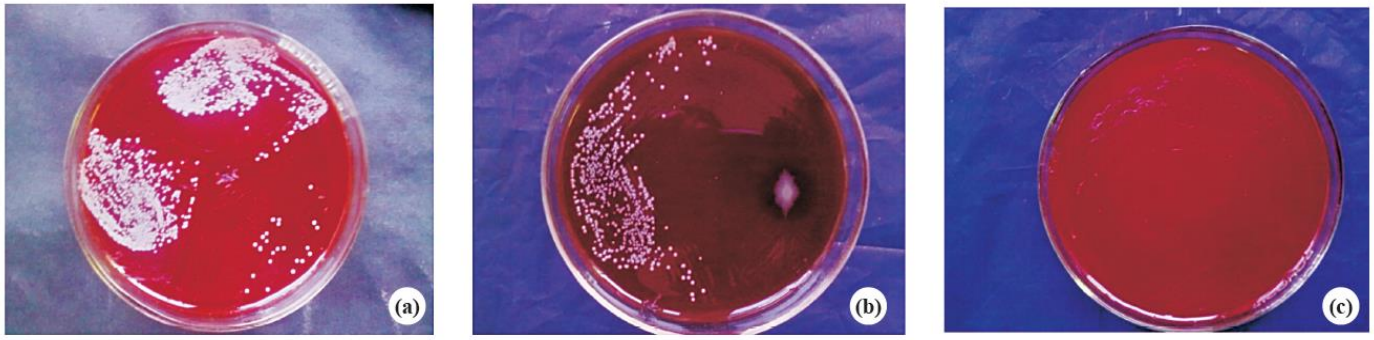
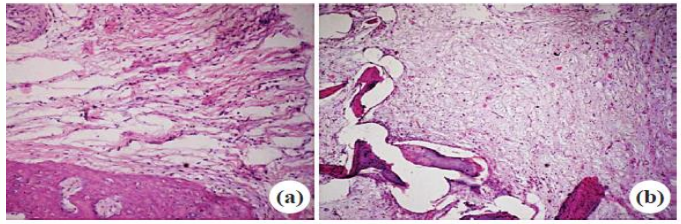


Fig.2. Pus culture showing positive result in 2nd **(a)** and 5th **(b)** week, negative result in 7th week **(c)** in group B rabbits.



4. Histopathological slides of group C rabbits showing minimal mmation at 3rd week **(a)** and no inflammation with new ıation at 6th week **(b)**. Stain H & E, magnification 10x.

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GEEPhage
Phagothérapie

In vivo efficacy of single phage versus phage cocktail in resolving burn wound infection in BALB/c mice

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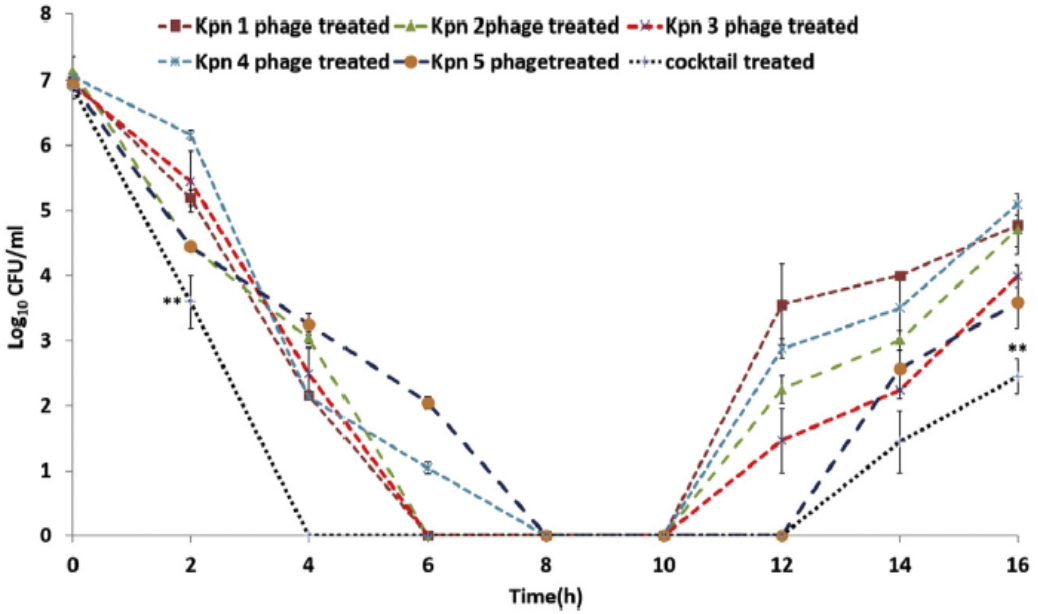

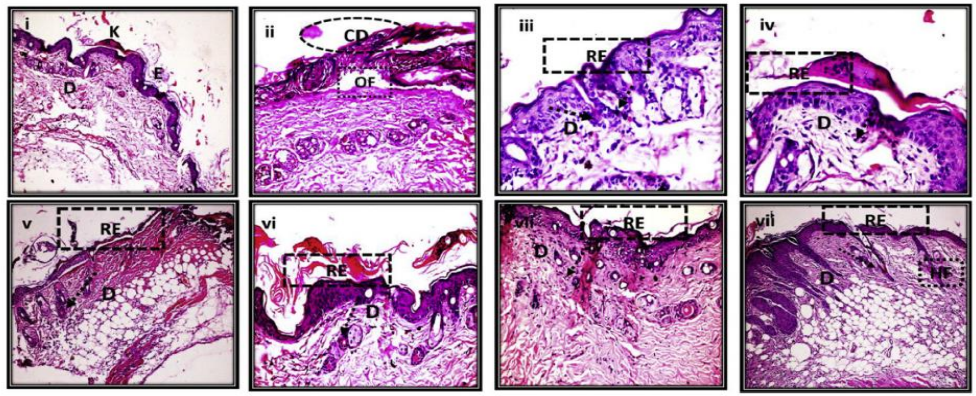

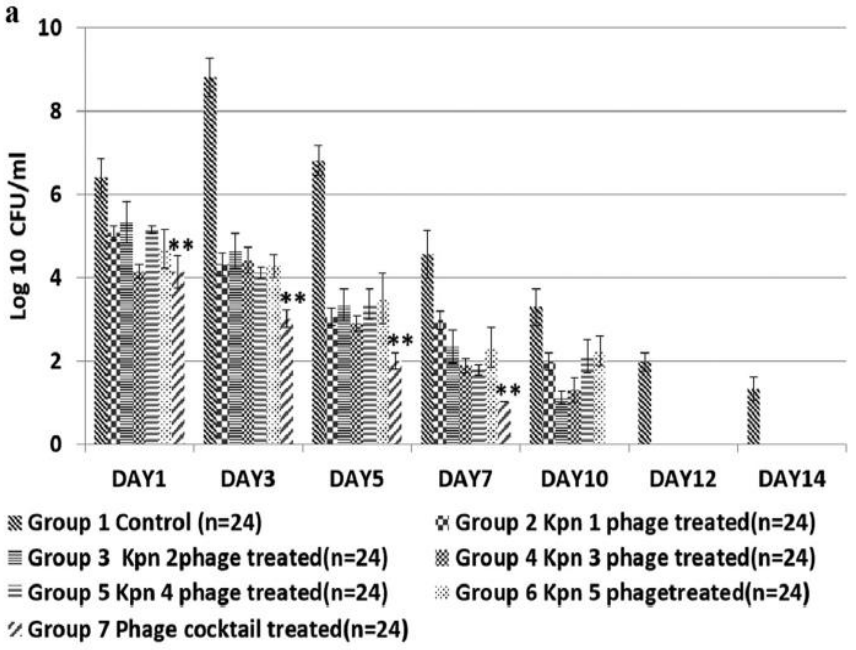

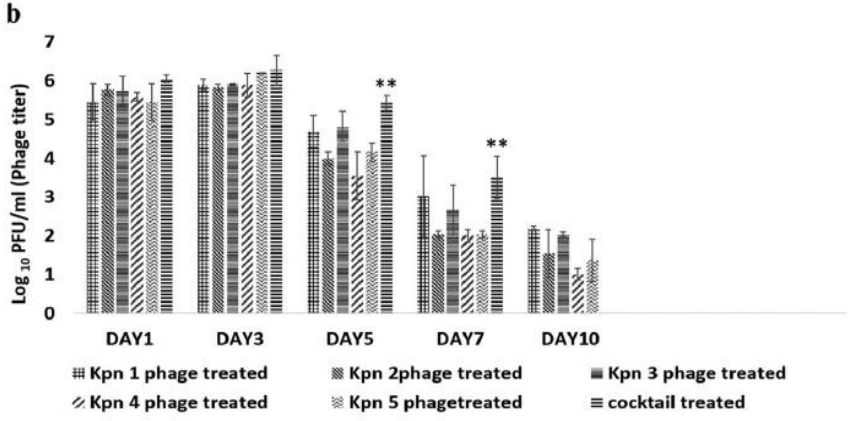

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F-274

Efficacy of a Bacteriophage Cocktail in a *Staphylococcus aureus* Mouse Pneumonia Model is Comparable to Vancomycin

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ABSTRACT

Background: A cocktail of four bacteriophages was constructed, which together has broad activity against a panel of clinically relevant and diverse *Staphylococcus aureus* isolates. The efficacy of this cocktail was evaluated vs. vancomycin (Van) in a *S. aureus* lung infection model.

Methods: Neutropenic ICR mice were inoculated intranasally (IN) with 6.98 log10 CFU in 50 µL of TSB. At 2 hrs postinfection, 50 µL phage cocktail was administered IN to three dosage groups (n=5) of mice consisting of $2X10^{10}$, $2X10^{9}$, and $2X10^{8}$ PFU/mL per phage. A second identical dose was administered IN at 6 hrs postinfection. The multiplicity of infection (MOI) of each of the 4 phages in the cocktail was ~ 50, ~5 and ~0.5 at the 2 hr time point (first administration). Van (100 mg/kg) was administered subcutaneously at 2 hr and 6 hr postinfection to a 4th group. The remaining groups were infected, but untreated. One group of control mice was euthanized at 2 hrs post-infection and all other mice were euthanized at 24 hrs and CFU/lung pair determined.

Results: Administration of phage cocktail resulted in *S. aureus* titers of 6.08, 6.16 and 7.8 mean log10 CFU/ lung pair for the $2X10^{10}$, $2X10^{9}$ and $2X10^{8}$ PFU/mL treatment groups, respectively. The two highest phage treatment groups and Van demonstrated a significant reduction ($p < 0.0001$) in lung CFU vs. the 24 hr nontreated control, with mean log10 CFU/lung pair decreases of 3.1 , 3.02, and 3.63 (Van), respectively. The $2X10^{8}$ PFU/mL treatment group did not achieve statistical significance vs. the 24 hr nontreated control ($p=0.074$). Twenty seven *S. aureus* colonies recovered from the murine lung infection model demonstrated similar sensitivity to the individual phages and to the 4 phage cocktail when compared to the starting strain.

Conclusions: The two highest doses of the phage cocktail exhibited efficacy that was comparable to Van in a *S. aureus* lung infection model. No evidence of recovery of phage resistant isolates was observed. These results demonstrate the potential therapeutic utility of phage therapy in bacterial lung infections.

BACKGROUND

- The rising tide of bacterial resistance to antibiotics has driven a renewed interest in novel therapies that can circumvent traditional mechanisms of resistance
- This clinical challenge has sparked a re-examination of the potential of bacteriophage (phage) therapy.

METHODS

Table 1. *S. aureus* Neutropenic Lung Model

Group	# Mice	Test Article	Route	Dose OR Titer (BID)	CFU Assessed (Time)
1	5	Phage	IN	$1x10^{8}$ PFU/phage	24 hr
2	5			$1x10^{9}$ PFU/phage	
3	5			$1x10^{7}$ PFU/phage	
4	5	Vancomycin	SC	100 mg/kg	24 hr
5	5	Untreated	–	–	24 hr
6	5		–	–	2 hr

- Mice were immunocompromised with 150 mg/kg cyclophosphamide on day -4 and 100 mg/kg on day -1
- Inoculum of 6.98 log_{10} CFU *S. aureus* UNT144-3 in 50 µL was delivered intranasally to female ICR mice
- Infection controls received 50 µL PBS-Mg diluent at 2 hrs and 6 hrs post infection
  - Mean bacterial lung titers were 7.24 log10 CFU/lung pair at 2 hrs
  - Increased to 9.18 log_{10} CFU/lung pair at 24 hrs
- A 4-phage cocktail was administered 2 hrs and 6 hrs post-infection using 3 dosing levels. 100 mg/kg vancomycin was administered SC at 2 hrs and 6 hrs post-infection
- 50 µL doses of phage mix were administered such that each mouse received $1X10^{9}$ PFU per phage, $1X10^{8}$ PFU per phage, or $1X10^{7}$ PFU per phage at each time point, according to its dosage group.
- At the time of the first administration of 50 µL phage mix, there were 1.74×10^{7} CFU/lung pair. Thus, the multiplicity of infection was ~50, ~5 and ~0.5 for the 3 dosage groups at the 2 hrs time point when the first phage mix dose was administered.

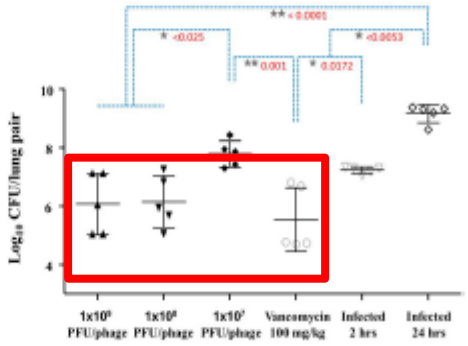
RESULTS

- In infected, untreated controls, the mean log CFU/lung pair titers increased ~ 2 logs between 2 hrs and 24 hrs post-infection **(Table 2)**.
- Administration of the two highest phage cocktail concentrations resulted in 3 log_{10} CFU reductions compared to the 24 hrs control group and 1 log_{10} CFU reductions compared to bacterial titers at 2 hrs post-infection

Table 2. Evaluation of 4 phage cocktail vs vancomycin

Dose OR Titer	CFU Assessed (Time)	Mean ± SD log_{10} CFU/ Lung Pair
$1x10^{8}$PFU/phage BID	24 hrs	6.08 ± 1.04
$1x10^{9}$ PFU/phage BID	24 hrs	6.16 ± 0.89
$1x10^{7}$ PFU/phage BID	24 hrs	7.8 ± 0.45
Vancomycin	24 hrs	5.55 ± 1.1
Infected, untreated	24 hrs	9.18 ± 0.32
Infected, untreated	2 hrs	7.24 ± 0.12

Figure 1. Efficacy of 4 phage cocktail in *S. aureus* lung model: statistical analysis of dosing groups



RESULTS

- **Figure 1** shows a comparison of the different treatment groups that demonstrated statistical significance as determined by ANOVA analysis (Tukey's multiple comparisons test).
- The $1x10^{8}$ PFU/phage and $1x10^{9}$ PFU/phage treatment groups demonstrated a significant reduction in lung CFU vs the 24 hrs non-treated control ($P < 0.0001$ for both). These two treatments were similar to the vancomycin-treated group.
- Although the $1x10^{7}$ PFU/phage treatment group also suggested a trend towards decreased lung counts, statistical significance vs the 24 hrs non-treated control was not achieved ($P=0.0745$).
- Twenty seven *S. aureus* colonies recovered from the murine lung infection model demonstrated similar sensitivity to the individual phages and to the 4-phage cocktail when compared to the starting strain.

CONCLUSIONS

- A *S. aureus* 4-phage cocktail administered at the two highest dosage levels demonstrated efficacy similar to vancomycin in a *S. aureus* lung model of infection.
- > 3 log reductions in mean CFU/lung pair were observed when compared to the 24 hrs untreated control, and > 1 log reductions were observed vs the 2 hrs untreated control.
- MOI values of ≥ 6 PFU per phage at the time of first administration were necessary for efficacy. An MOI of 0.6 was ineffective.
- These data support the potential utility of phage cocktails as a therapeutic agent for *S. aureus* lung infections.

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We thank K. Božnik, Z.Kalcic and A. Alegro for technical assistance in phage production.

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Bacteriophage therapy for the treatment of *P. aeruginosa* infections in cystic fibrosis patients

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INTRODUCTION

Chronic lung infections caused by *Pseudomonas aeruginosa* (PA) are a major cause of morbidity and mortality in cystic fibrosis (CF) patients. In some cases effective antibiotic therapy is no longer available, with multi-drug resistant (MDR) forms of these bacteria becoming increasingly challenging to treat. Thus, new alternative means of controlling MDR PA infections are urgently needed. Bacteriophage (phage) therapy is a potential therapeutic tool for the treatment of bacterial infections. However, due to the specific nature of phages, questions have been raised about the clinical practicality of bacteriophage based products and their ability to be effective against a range of clinical isolates.

We have previously reported in the development of three prototype phage mixes and shown that phages are efficacious in reducing both bacterial load and inflammation in a murine lung infection model [1]. In this study, we have expanded the in vitro testing and developed a bacteriophage mix (AB-PA01) active against relevant clinical PA isolates collected from around the world. In addition, we demonstrated the efficacy of AB-PA01 in vivo in a murine lung infection model.

METHODS

In vitro testing: Lytic bacteriophages were isolated from environmental sources in Australia and England and their activity screened against a reference collection of 67 *P. aeruginosa* from CF patients. A prototype combination of four phages was then developed and tested for its activity against 429 global CF and non-CF clinical isolates collected between 2007 to 2015. Isolates included were both antibiotic susceptible/resistant and mucoid/non-mucoid.

In vivo testing: Immunocompetent CD-1 female mice were inoculated intranasally (IN) with 6.26 $\log_{10}$ CFU in 50 µL of TSB. At 2 hrs post-infection (PI), 50 µL 4-phage mix was administered IN to three dosage groups (n=5) of mice consisting of $7.5x10^9$, $7.5x10^8$, and $7.5x10^7$ PFU/mL per phage (for a total of $1.5x10^9$, $1.5x10^8$, or $1.5x10^7$ PFU administered). A second identical dose was administered IN at 6 hrs PI. Meropenem (25 mg/kg) was administered subcutaneously at 2 hrs and 6 hrs PI to a fourth group. A fifth group was infected, but treated with the phage diluent. All mice were euthanized at 24 hrs and CFU/lung pair determined. Statistical analysis was performed using Tukey's multiple comparisons test (Graphpad Prism 6, $p < 0.05$).

RESULTS

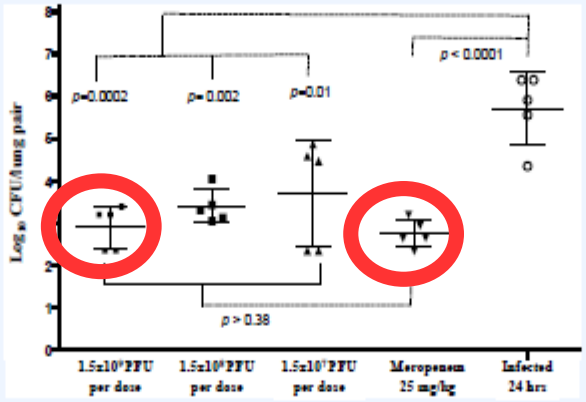
AB-PA01 Phage Mix Host Range

Phages were isolated from a variety of environmental sources in Australia and the UK, using different protocols as previously described [2]. Four phages were selected based on the spectrum of activity against a reference panel of 67 distinct CF isolates, with the number of isolates targeted by ≥2 phages considered an important selection criteria. The overall activity of the selected 4-phage mix **AB-PA01** is summarised in Table 1.

Number of Isolates	Area (Year of Isolation)	Type	AB-PA01 % of Activity	% of Isolates sensitive to ≥2 phages in mix
67*	UK/AU/USA (2012-13)	CF	95.5%	87.5%
120	UK (2012-13)	CF	85.0%	95.0%
60	AU (2007-2013)	CF	93.3%	98.2%
40	USA (2014)	CF	87.5%	91.4%
82	UK (2015)	CF	81.7%	92.5%
60	USA/EU/AU (2013)	Non-CF	83.3%	80.0%
369	Total CF Isolates		87.8%	93.2%
429	Total Isolates (CF + Non-CF)		87.2%	91.4%

Table 1 Activity of AB-PA01 against global CF and non-CF *P. aeruginosa* isolates. Phage mix (10^8 PFU/ml) was used to spot onto *P. aeruginosa* lawns. Each strain was considered sensitive if more than 20 plaques were observed. *Reference panel used for phage selection composed of 67 distinct CF strains that included well characterized CF epidemic clones. UK: United Kingdom; AU: Australia; USA: The United States of America. % of activity=[no. sensitive isolates/total no. of isolates tested] x 100

Murine Lung Infection Model



CONCLUSIONS

- Four phages were isolated and combined into a effective prototype phage mix capable of infecting *P. aeruginosa* clinical isolates collected around the world. The developed 4-phage mix was shown to infect both antibiotic susceptible/resistant and mucoid/non-mucoid CF strains.
- This study has shown that AB-PA01 has a broad range of activity addressing concerns that the specificity of phages could make this therapy impractical in the clinical environment. However, it is likely that, like the flu vaccines, these broad spectrum preparations will need to be reformulated overtime as the bacterial populations evolve.
- AB-PA01 administered at the three dosage levels demonstrated efficacy similar to meropenem in a *P. aeruginosa* murine lung model of infection. However, there seems to be a non-significant trend suggesting a possible dose-dependent effect.
- In addition, we have confirmed the usability of AB-PA01 for:
  - ✓ Clinical use (exclusively lytic, efficacious in vivo)
  - ✓ Nebulisation (no significant decreases in titre were observed)
  - ✓ GMP Manufacturing (long-term stability, current process optimisation)

The use of phages as therapeutic tools continues to be a viable option for the treatment of PA infections in CF patients. AmpliPhi Biosciences, in collaboration with the Brompton Hospital, plan to evaluate the safety and efficacy of AB-PA01 in CF patients.

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Service de santé des armées

Votre vie, notre combat

SEVENTH FRAMEWORK PROGRAMME

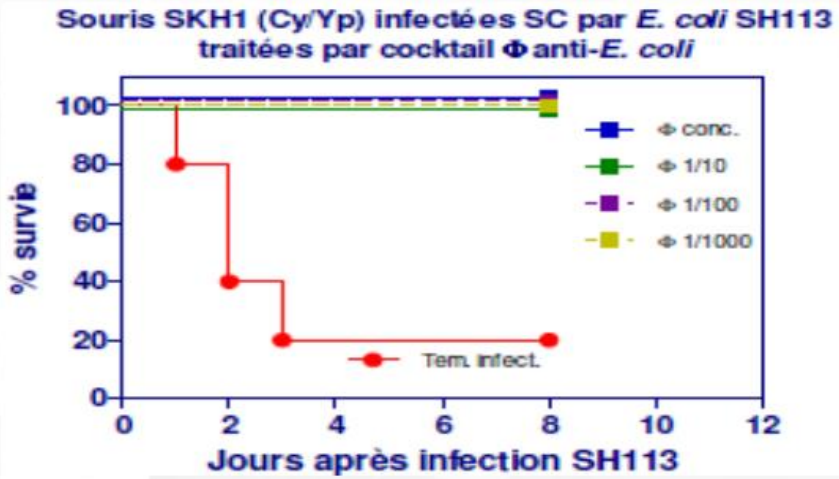
PhagoBurn

BURNED AND INFECTED MICE

Jour	-3	-2	-1	0	1	2	3	4
	1,5mg Cy	Burn	1,5mg Cy	Infection	1,5mg Cy			
Mode injection	IP	Yperite	IP	**SC** 10^7cfu	IP			
PHAGE				SC 6h post-infection				

Conclusions PP0121

- No treatment Survival rate : 20%
- Treated J0 (infection+6h) via SC :SR = 100%
- Dilution of cocktail = 10^5 PFU



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RÉPUBLIQUE FRANÇAISE
MINISTÈRE DE LA DÉFENSE

P. Jault

Efficacy of lytic *Staphylococcus aureus* bacteriophage against multidrug-resistant *Staphylococcus aureus* in mice

Joseph Michael Ochieng' Oduor[1,2], Nyamongo Onkoba[3], Fredrick Maloba[4], Washingtone Ouma Arodi[2] Atunga Nyachieo[1,5]

Abstract

Introduction: The use of bacteriophages as an alternative treatment method against multidrug-resistant bacteria has not been explored in Kenya. This study sought to determine the efficacy of environmentally obtained lytic bacteriophage against multidrug-resistant *Staphylococcus aureus* (MDRSA) bacterium in mice.

Methodology: *Staphylococcus aureus* bacterium and *S. aureus*-specific lytic phage were isolated from sewage and wastewater collected within Nairobi County, Kenya. Thirty mice were randomly assigned into three groups: MDRSA infection group (n = 20), phage-infection group (n = 5), and non-infection group (n = 5). The MDRSA infection group was further subdivided into three groups: clindamycin treatment (8 mg/kg; n = 5), lytic phage treatment (10^8 PFU/mL (n = 5), and a combination treatment of clindamycin and lytic phage (n = 5). Treatments were done at either 24 or 72 hours post-infection (p.i), and data on efficacy, bacterial load, and animal physical health were collected.

Results: Treatment with phage was more effective (100%) than with clindamycin (62.25% at 24 hours p.i and 87.5% at 72 hours p.i.) or combination treatment (75% at 24 hours p.i. and 90% at 72 hours p.i.) ($p < 0.001$).

Conclusions: The results show that the environmentally obtained *S. aureus* lytic bacteriophage has therapeutic potential against MDRSA bacterium in mice.

Table 3. End point (day 10) bacteria count (mean log10 CFU/g ± SE) isolate from organs.

Groups	Mean $\log_{10}$ CFU/mL ± SE at day 10 p.i		Mean $\log_{10}$ CFU/g ± SE at day 10 p.i		
	Treatment at 24 hours post infection	Treatment at 72 hours post infection	Brain	Lungs	Liver
Naive	0.0	0.0	0.0	0.0	0.0
Control phage	0.0	0.0	0.0	0.0	0.0
MDRSA only	8.0 ± 0.2	9.0 ± 0.2	7.2 ± 0.2	7.0 ± 0.2	9.0 ± 0.2
MDRSA + clindamycin	3.0 ± 0.2 (**62.25%**)	1.0 ± 0.2 (**87.5%**)	3.0 ± 0.2	1.4 ± 0.2	1.6 ± 0.2
MDRSA + phage	0.0 (**100%**)	0.0 (**100%**)	0.0	0.0	0.0
MDRSA + clindamycin + phage	2.0 ± 0.2 (**75%**)	0.0 (**100%**)	0.0	4.0 ± 0.2	2.0 ± 0.2

MDRSA: multidrug-resistant *Staphylococcus aureus*; p.i.: post-infection.

Figure 2. Survival rates.

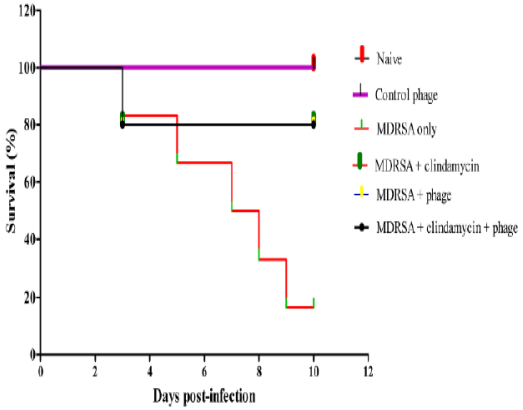
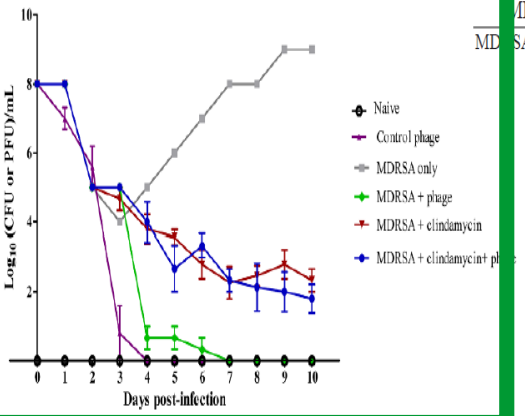


Figure 3. Blood bacteremia and viremia levels of mice.



Prospects of Phage Application in the Treatment of Acne Caused by *Propionibacterium acnes*

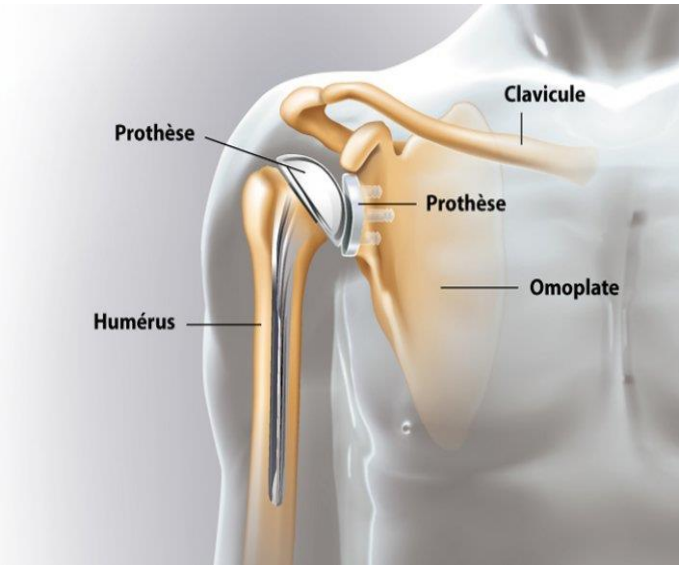
Ewa Jończyk-Matysiak[1], Beata Weber-Dąbrowska[1,2], Maciej Żaczek[1], Ryszard Międzybrodzki[1,2,3], Sławomir Letkiewicz[2,4], Marzanna Łusiak-Szelchowska[1] and Andrzej Górski[1,2,3]*

frontiers in Microbiology

TABLE 2 | Described phages that are active against *Propionibacterium acnes*.

Phage symbol, total number of isolated phage strains	Classification in family	Brief characteristics	Host range and specificity of action	Possible use in phage therapy
PA6	*Siphoviridae*	Lytic (lack of lysogeny genes). Phage isolated from skin scrub wash sample from patient. Phage produces clear plaques with turbid centers.	Able to lyse *P. acnes* strains, but not able to lyse other strains that are a part of the skin microbiome: *Propionibacterium granulosum*, *Propionibacterium avidum*, *Staphylococcus epidermidis*, Corynebacterium bovis (Farrar et al., 2007)	High specificity only against *P. acnes* and lytic life cycle may predispose to use of this phage in the therapy of acne.
PAC1-PAC10	Not done	Pseudolysogenic life cycle.	Lysis of *P. acnes* strains, but not lysis of *P. acidipropionici*, *P. avidum*, *P. cyclohexanicum*, *P. jensenii*, *P. thoenii*, *P. freudenreichii*. Narrow lytic spectrum.	In Cetomacrogol cream aqueous concentration of phage for potential application in topical treatment of acne (Brown et al., 2016).
		Phage isolated from bacteria from skin swab sample from patient. It did not contain bacterial virulence factors.		
PAD2-PAD48, PAS2-PAS52	*Siphoviridae*	Presence of pseudolysogeny. Do not confer superinfection immunity.	Species specific. Only infect *P. acnes*, not other strains closely related to *Propionibacterium* (Lood et al., 2008).	Probably bad candidate for phage therapy.
P1.1, P9.1, P14.4, P100A, P100D, P100.1, P101.A, P104.A, P105	*Siphoviridae*	Probably the presence of pseudolysogeny (lack of lysogeny-related genes) phages. Isolated from healthy subjects and patients with acne. Lack of genetic diversity.	Broad range of clinical isolates, phage immunity if present is connected with the presence of chromosomally encoded elements.	Marinelli et al. (2012) suggested use of endolysin—peptidoglycan hydrolases that are bacteriophage-encoded antimicrobial peptides to treat bacterial infection.
48 phages, e.g., PHL111M01, PHL071N05, PHL060L00, PHL073M02	*Siphoviridae*	Pseudolysogenic and/or life cycle. 21 phages were isolated from patients with acne, 27 from healthy volunteers. Phages have limited diversity in genome.	*P. acnes* strains (including clades IA-1, IA-2, IB-1, and IB2) were susceptible to all 15 tested phages. But strains of clade IB-3, II, II were highly resistant to phages. Moreover, *P. granulosum* and *P. avidum* were resistant to all tested phages. Two strains of *P. humerusii* were susceptible to all tested phages, one was susceptible to 10 from 15 phages. Activity of *P. acnes* phages includes bacterial strains that are closely related to *Propionibacterium* species.	The authors suggested that the isolated phages may be used in modulation of *Propionibacterium* populations in human skin (Liu et al., 2015).
9 phages: from P-a-1 to P-a-9	Not done	Lytic phages, lysogenic ones were not detected. P-a1 to P-a7 were isolated from plaques on *P. acnes* lawn, but P-a-8 and p-a-9 came from sewage.	Both Gram-positive and Gram-negative strains from genera other than *Propionibacterium* were not lysed by these bacteriophages (Zierdt, 1974).	Bacteriophages were used to distinguish *C. anoes* from *C. avidum* and *C. granulosum*. Lytic life cycle may predispose to use of this phage in the therapy of acne.
15 phages	Polyhedral heads with flexible unsheathed tails	Lysogenic, induced with mitomycin C from 17% of *P. acnes* strains	Isolated from *P. acnes* of healthy individuals. Strains of *P. acnes* belonging to serotype I were more susceptible to phage than those from serotype II (Webster and Cummins, 1978).	Probably bad candidate for phage therapy because of lysogenic cycle.
12 phages	Not done	Lysogenic phages. Phages isolated from skin swab sample from patient.	Phage of varying host range, but none which lyses all subtypes of *P. acnes*. (Neely et al., 2008).	Bad candidate for phage therapy because of lysogenic cycle.

Propionibacterium acnes is associated with purulent skin infections, and it poses a global problem for both patients and doctors. Acne vulgaris (acne) remains a problem due to its chronic character and difficulty of treatment, as well as its large impact on patients' quality of life. Due to the chronic course of the disease, treatment is long lasting, and often ineffective. Currently there are data regarding isolation of *P. acnes* phages, and there have been numerous studies on phage killing of *P. acnes*, but no data are available on phage application specifically in acne treatment. In this review, we have summarized the current knowledge on the phages active against *P. acnes* described so far and their potential application in the treatment of acne associated with *P. acnes*. The treatment of acne with phages may be important in order to reduce the overuse of antibiotics, which are currently the main acne treatment. However, more detailed studies are first needed to understand phage functioning in the skin microbiome and the possibility to use phages to combat *P. acnes*.



REVIEW
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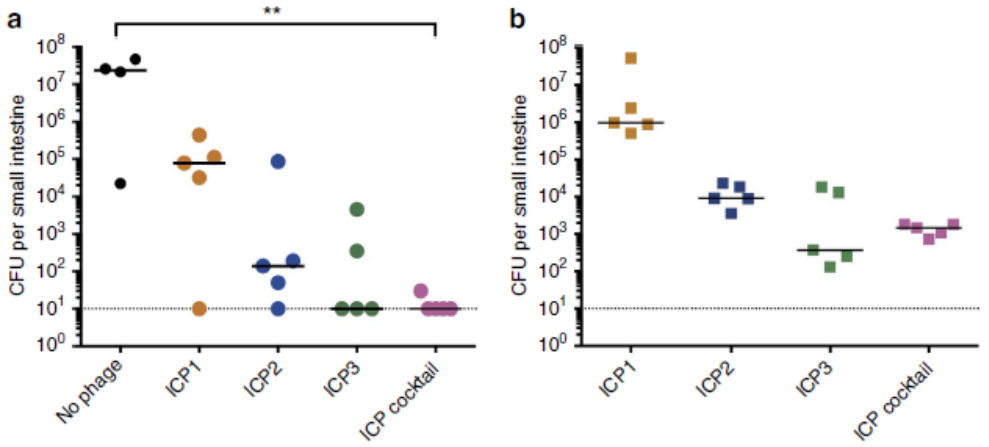
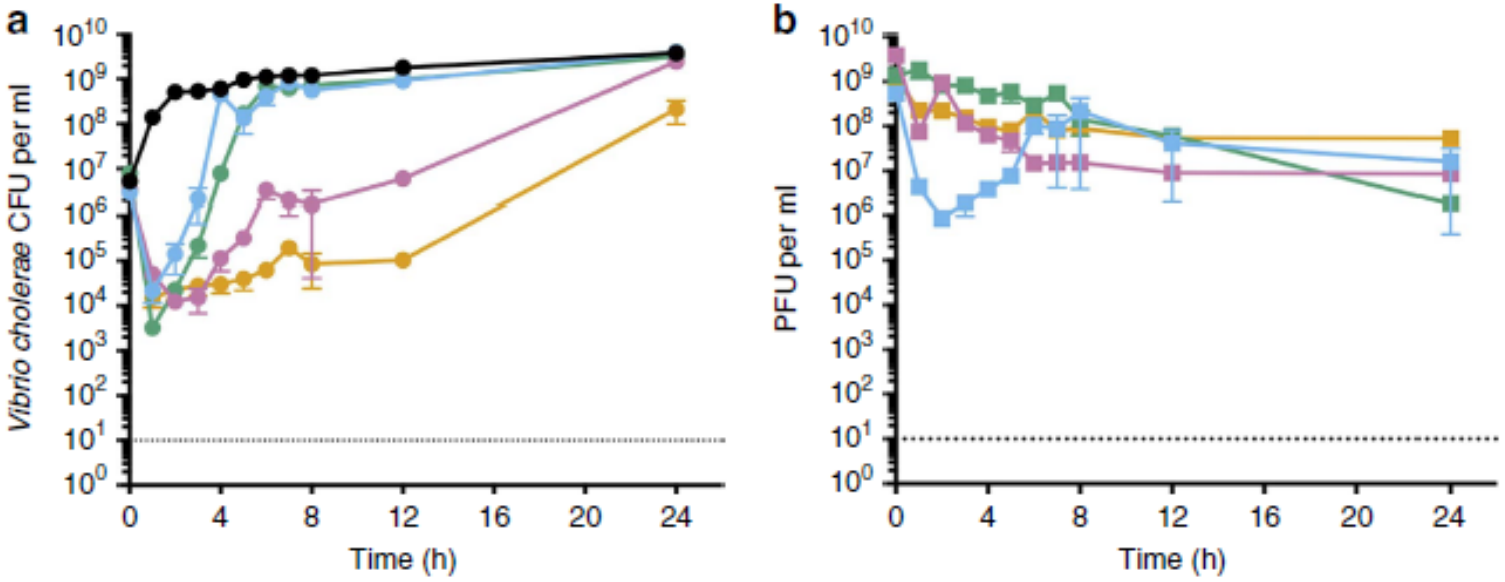
CENTRE HOSPITALIER INTERCOMMUNAL
VILLENEUVE-SAINT-GEORGES
Lucie & Raymond AUBRAC

A cocktail of three virulent bacteriophages prevents *Vibrio cholerae* infection in animal models

Minmin Yen[1,2,*], Lynne S. Cairns[1,*] & Andrew Camilli[1]

Effective prevention strategies will be essential in reducing disease burden due to bacterial infections. Here we harness the specificity and rapid-acting properties of bacteriophages as a potential prophylaxis therapy for cholera, a severely dehydrating disease caused by *Vibrio cholerae*. To this end, we test a cocktail of three virulent phages in two animal models of cholera pathogenesis (infant mouse and rabbit models). Oral administration of the phages up to 24 h before *V. cholerae* challenge reduces colonization of the intestinal tract and prevents cholera-like diarrhea. None of the surviving *V. cholerae* colonies are resistant to all three phages. Genome sequencing and variant analysis of the surviving colonies indicate that resistance to the phages is largely conferred by mutations in genes required for the production of the phage receptors. For acute infections, such as cholera, phage prophylaxis could provide a strategy to limit the impact of bacterial disease on human health.





Gastroenterol Hepatol Bed Bench. 2017 Spring;10(2):131-136.

Phage therapy: assessment of the efficacy of a bacteriophage isolated in the treatment of salmonellosis induced by Salmonella enteritidis in mice.

Nikkhahi F[1], Soltan Dallal MM[1,2], Alimohammadi M[3], Rahimi Foroushani A[4], Rajabi Z[2], Fardsanei F[1], Imeni SM[2,5], Torabi Bonab P[2].

Author information

Abstract

AIM: This work aims to isolate and perform comparative studies of a phages active against a Salmonella enteritidis strain from Iran. Also, suitable phage candidates for therapy of mice will be selected.

BACKGROUND: Bacteriophage is of particular interest as a biocontrol agent in the prevention of food-borne illnesses. In recent years tend to use bacteriophages to control pathogenic bacteria has increased. A bacteriophage is considered to be a potent antibiotic alternative for treating bacterial infections.

METHODS: the specific phages against Salmonella Enteritidis was isolated and candidates for therapy of mice will be selected. Mouses divided into the six specific groups. Groups of mice were as follows: A: Bacteri (control) B: Bacteri+ bacteriophage (Simultaneous), C: Bacteri + bacteriophage Four days later, D: Bacteriophage + bacteri four days later E: Bacteri+ Ciprofloxacin (Simultaneous) F: Bacteri+ ciprofloxacin+ bacteriophage (Simultaneous).

RESULTS: In this study, a lytic bacteriophage is isolated and it shows that phage has a head size of 46 nm and without a tail, by using an electron microscope. Oral administration of a single dose of 2 × 109 PFU/mouse bacteriophage enable to protect mouse against salmonellosis and it causes treatment of salmonellosis in mice.

CONCLUSION: The use of this phage compared to ciprofloxacin shows that in addition of the treatment of mouse, it also prevents weight loss.

KEYWORDS: Bacteriophage; Isolation; Salmonella enteritidis

PMID: 28702137

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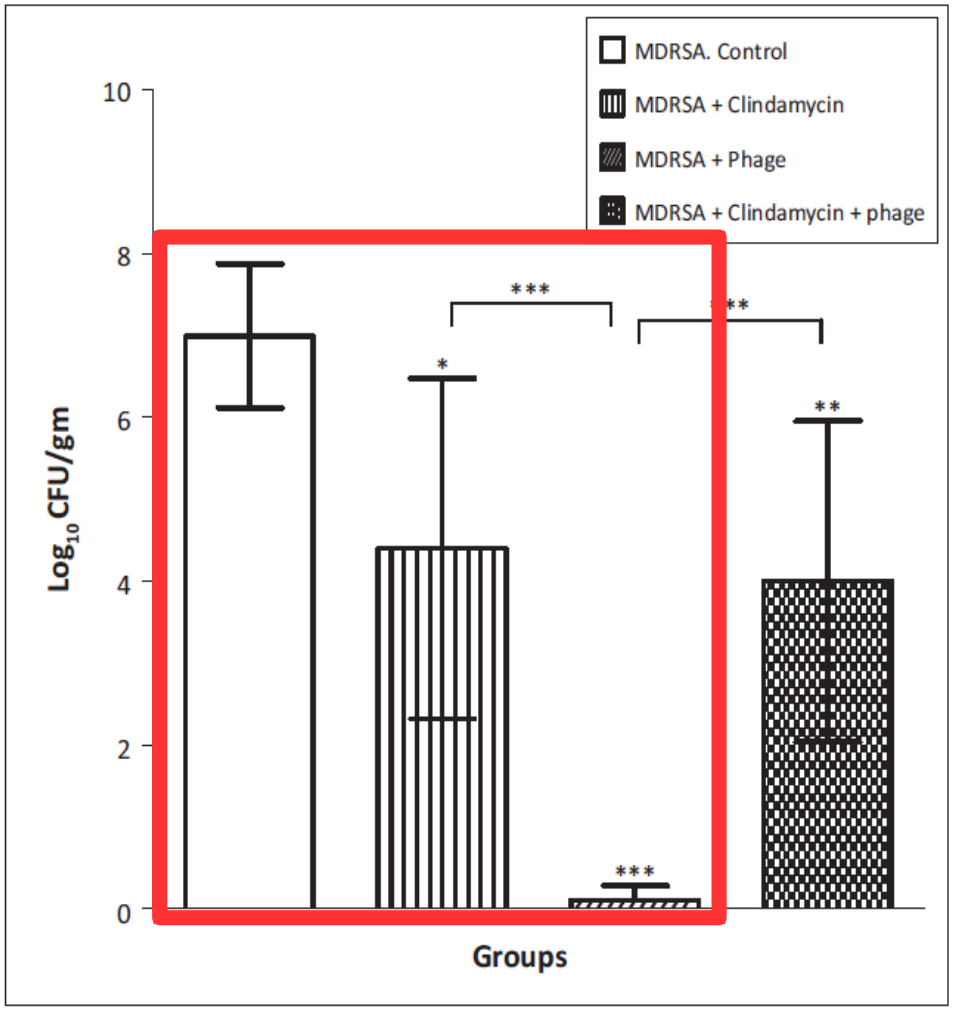
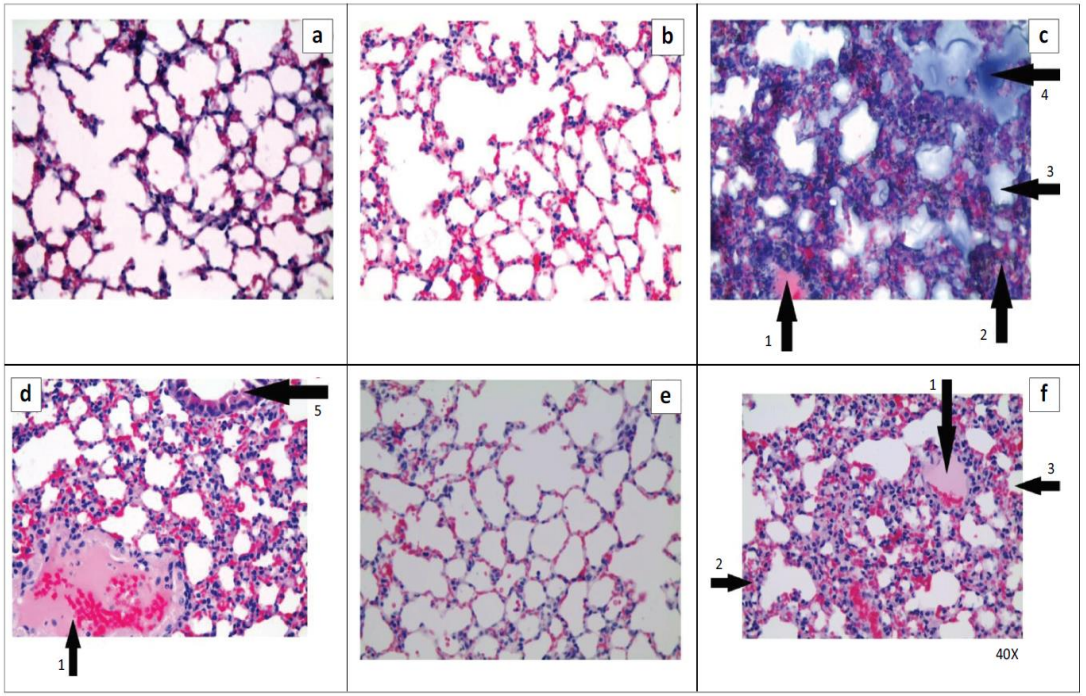
Experimental phage therapy against haematogenous multi-drug resistant *Staphylococcus aureus* pneumonia in mice



MDRSA, multidrug-resistant *Staphylococcus aureus*.



MDRSA, multidrug-resistant *Staphylococcus aureus*.

FIGURE 5: Mouse lung tissue slides (40x magnification). Tissue from non-infected, non-treated mice (A) and phage-infected, non-treated mice (B) were well ventilated. Tissue from MDRSA-infected, non-treated control mice (C) had pockets of serous fluid (pneumonia) (1), lymphocyte-infiltrated (inflamed) septa (2), deflated alveoli (3) and alveoli full of mucus (4). Tissue from MDRSA-infected, clindamycin-treated mice (D) had pockets of pneumonia (1), inflamed septa and perivascular fibrosis of blood vessels (5). Tissue from MDRSA-infected, phage-treated mice (E) was well ventilated with resolved inflammation. Tissue from MDRSA-infected mice treated with a combination of clindamycin and phage (F) had pockets of pneumonia (1), inflamed septa (2) and deflated alveoli (3).

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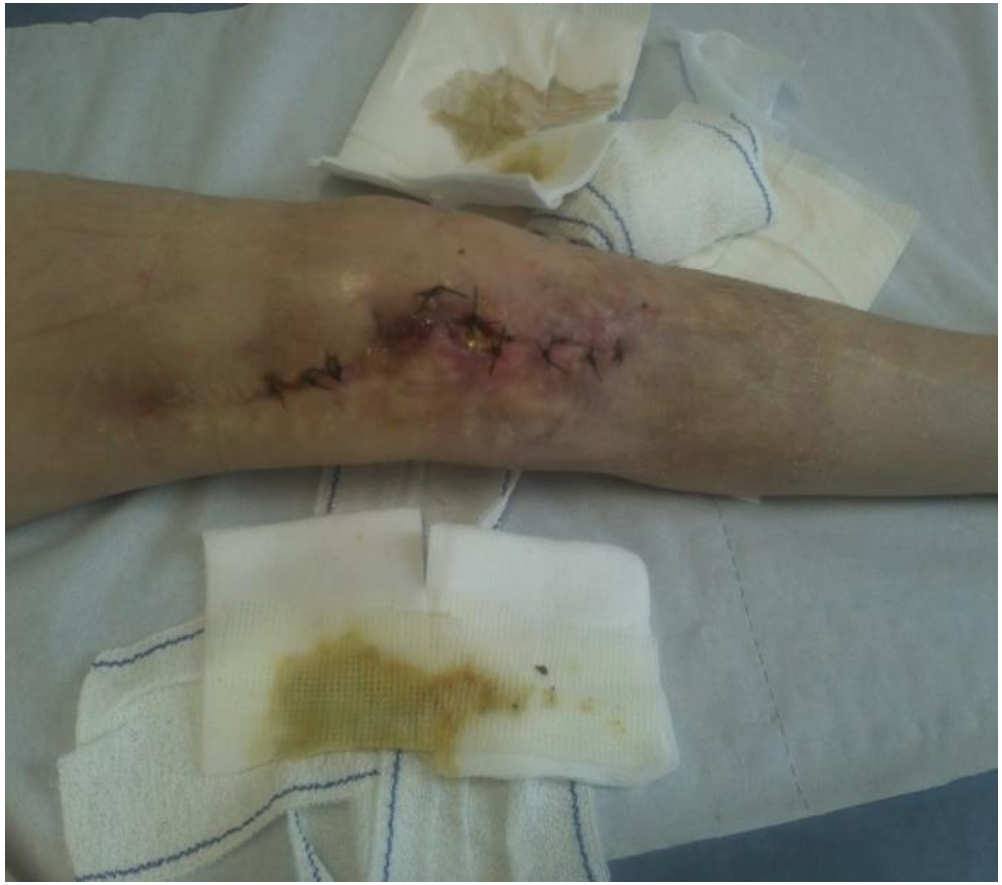
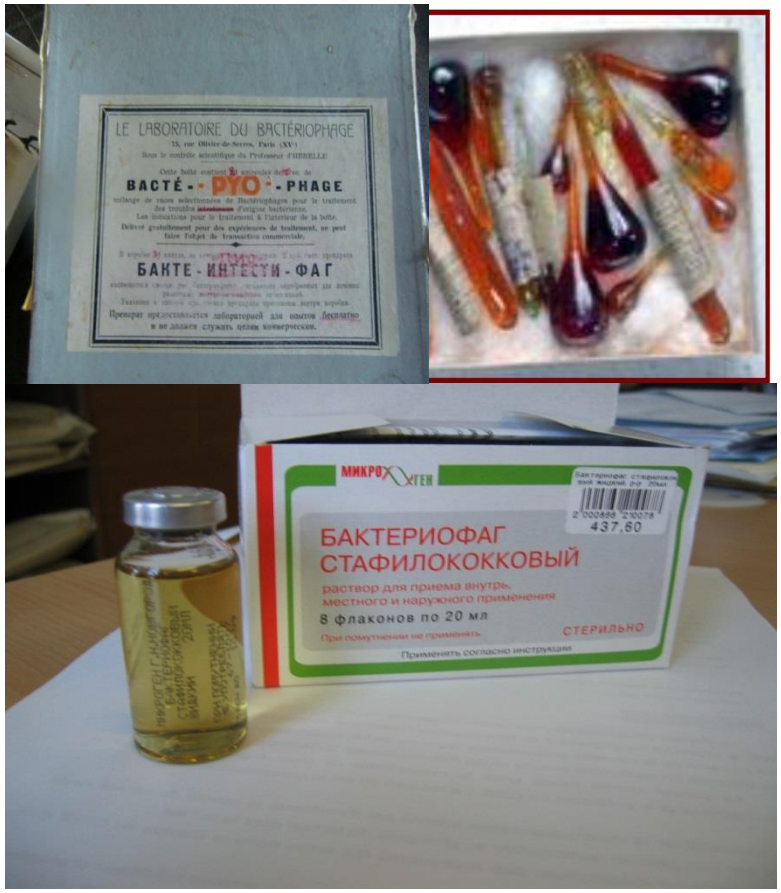
GEEPhage
Phagothérapie

LES BACTERIOPHAGES CHEZ L'HOMME





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Lucie & Raymond AUBRAC

GEEPhage
Phagothérapie

PRODUCTION DE PHAGES

Institute ELIAVA

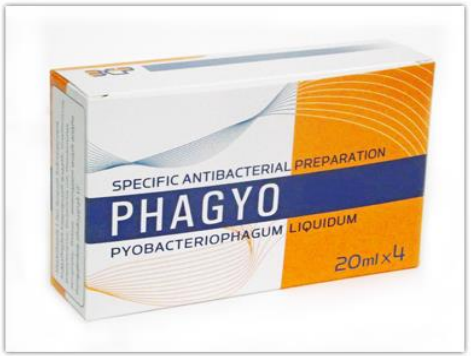
Staphylococcus phage for intravenous use


INTESTI
BACTERIOPHAGE
ANTIBACTERIAL SOLUTION FOR ORAL USE
10 ml x 5
Sterile filtrate of phage lysates
Staphylococcus, Enterococcus, Proteus, Shigella,
Salmonella, Escherichia coli, Pseudomonas aeruginosa
Preparations developed by
GEORGE ELIAVA INSTITUTE
OF BACTERIOPHAGES, MICROBIOLOGY AND VIROLOGY
FERSISI
BACTERIOPHAGE
ANTIBACTERIAL SOLUTION FOR TOPICAL AND ORAL USE
10 ml x 5
Phages active against: (pfu/ml)
Staphylococcus (10⁹), Streptococcus (10⁹),
Preparations developed by
GEORGE ELIAVA INSTITUTE
OF BACTERIOPHAGES, MICROBIOLOGY AND VIROLOGY
Preparations developed by
GEORGE ELIAVA INSTITUTE
OF BACTERIOPHAGES, MICROBIOLOGY AND VIROLOGY
STAPHYLOCOCCAL
BACTERIOPHAGE
STERILE PURIFIED SOLUTION FOR ORAL AND TOPICAL USE
10 ml x 5
PYO
BACTERIOPHAGE
ANTIBACTERIAL SOLUTION FOR TOPICAL AND ORAL USE
10 ml x 5
Sterile filtrate of phage lysates
Staphylococcus, Streptococcus, Proteus,
Escherichia coli, Pseudomonas aeruginosa
Preparations developed by
GEORGE ELIAVA INSTITUTE
OF BACTERIOPHAGES, MICROBIOLOGY AND VIROLOGY
SES
BACTERIOPHAGE
ANTIBACTERIAL SOLUTION FOR TOPICAL AND ORAL USE
10 ml x 5
Phages active against: (pfu/ml)
Staphylococcus (10⁹), Streptococcus (10⁹), Escherichia coli (10⁹)
Preparations developed by
ENKO
BACTERIOPHAGE
ANTIBACTERIAL SOLUTION FOR TOPICAL AND ORAL USE
10 ml x 5
Phages active against: (pfu/ml)
Staphylococcus (10⁹), Shigella (10⁹), Salmonella (10⁹), Escherichia coli (10⁹),
Preparations developed by
GEORGE ELIAVA INSTITUTE

Manufacturer:
JSC Biochimpharm
Addres: Gotua str. 3, Tbilisi 0160, Georgia.
Phone: +995 32 2 244777; +995 32 2 244778
Fax: +995 32 2 380895
E-mail: biochimpharm@geophage.ge
Web-site: www.biochimpharm.ge

State Registration No R-001875
Renewal: March 2010

Approved by Order of the Head of Drug Agency of the Ministry of Labour, Health and Social Affairs of Georgia № 40/adm, January 29, 2008



Quantitative composition
PHAGESTI is presented as a liquid and 1 ml product contains:
Bacteriophagum Shigella;
Bacteriophagum Salmonella;
Bacteriophagum E.Coli;
Bacteriophagum Proteus (vulgaris, mirabilis);
Bacteriophagum Staphylococcus;
Bacteriophagum Pseudomonas;
Bacteriophagum Enterococcus;
Each quantity not less than - 10^5;

Recommendation dosage scheme for treatment:

Age	Per oral administration	Per rectum (enema)
up to 6 month	5 ml X 1a day	5 ml X 1 a day
6 to 12 month	5 ml X 2 a day	10 ml X 1 a day
1 to 3 years	5 ml X 3 a day	20 ml X 1 a day
3 to 8 years	10 ml X 2-3 a day	30 ml X 1 a day

MICROGEN

Bacteriophages

- *Pseudomonas aeruginosa bacteriophage*
- *Coliproteic bacteriophage*
- *Staphylococcal bacteriophage*
- *Dysenteric bacteriophage, polyvalent*
- *Klebsiella bacteriophage, polyvalent, purifi...*
- *Klebsiella pneumoniae bacteriophage, purified*
- *Klebsiphage (Klebsiella pneumoniae bacteriophage)*
- *Coli bacteriophage*
- *Proteus bacteriophage*
- *Salmonellosis of ABCDE groups bacteriophage*
- *Streptococcal bacteriophage*
- *Intesti-bacteriophage*
- *Pyopolyphage (pyobacteriophage combined, liquid)*
- *Pyobacteriophage polyvalent, purified®*
- *SEXTAPHAGE ® (pyobacteriophage polyvalent)*

INTESTI-BACTERIOPHAGE

solution for oral and rectal use

Mixture of filtrates of phagolysates active against Shigella flexneri 1, 2, 3, 4, 6 serovars and Zonnei, salmonellae of paratyphoid A and B, typhimurium, cholerae suis, infantum, oranienburg, enteritidis; enteropathogenic colibacillus of etiologically significant serovariants, enterococci, staphylococci, Pseudomonas aeruginosa, Proteus vulgaris and mirabilis. Intesti-bacteriophage possesses the ability to specifically lyse the above-mentioned bacteria.

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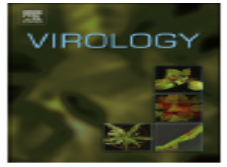

Safety analysis of a Russian phage cocktail: From MetaGenomi... analysis to oral application in healthy human subjects

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

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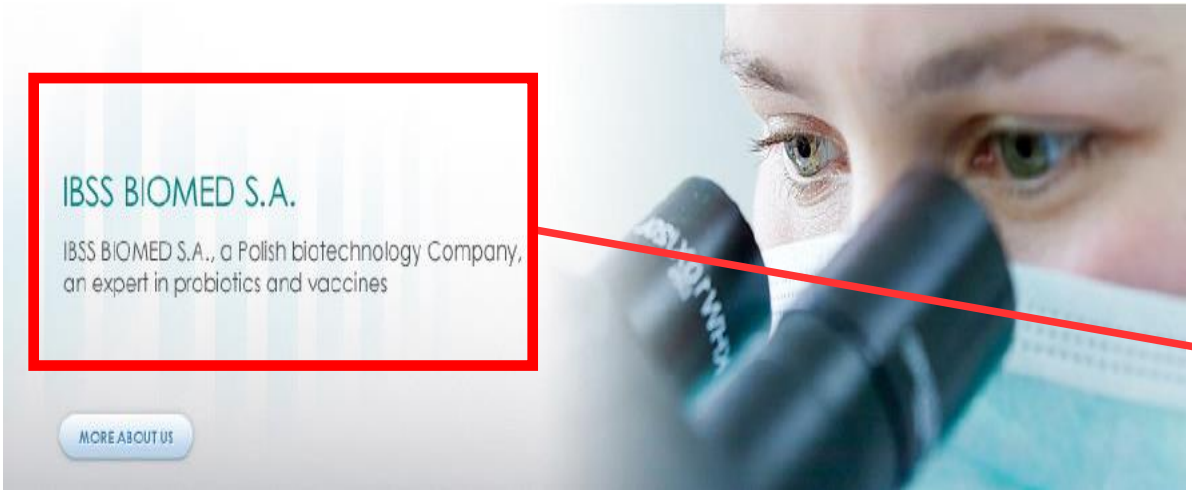
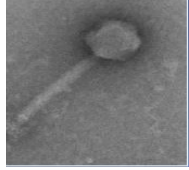
VILLENEUVE-SAINT-GEORGES
Lucie & Raymond AUBRAC



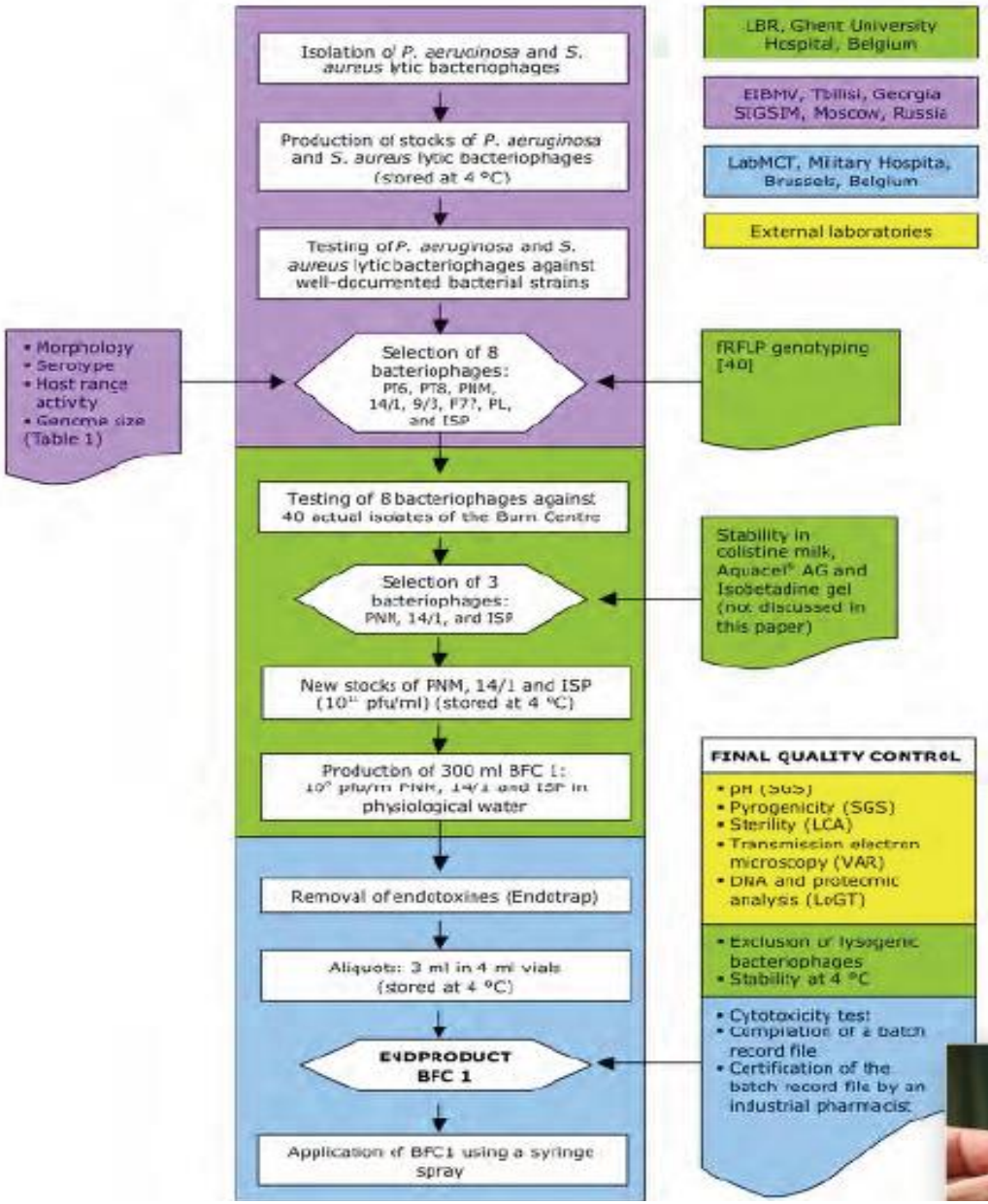
The GMP (Good Manufacturing Practice) System that is in place at the company guarantees that the products manufactured by IBSS BIOMED S.A. fully meet quality and safety standards.

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"

Quality and Safety Requirements for Sustainable Phage Therapy Products

Jean-Paul Pirnay • Bob G. Blasdel • Laurent Bretaudeau • Angus Buckling • Nina Chanishvili • Jason R. Clark • Sofia Corte-Real • Laurent Debarbieux • Alain Dublanchet • Daniel De Vos • Jérôme Gabard • Miguel Garcia • Marina Goderdzishvili • Andrzej Górski • John Hardcastle • Isabelle Huys • Elizabeth Kutter • Rob Lavigne • Maia Merabishvili • Ewa Olchawa • Kaarle J. Parikka • Olivier Patey • Flavie Pouilot • Gregory Resch • Christine Rohde • Jacques Scheres • Mikael Skurnik • Mario Vaneechoutte • Luc Van Parys • Gilbert Verbeken • Martin Zizi • Guy Van den Eede

Table I Expert Consensus Quality and Safety Requirements for Sustainable Phage Therapy Products

A. Production environment

When production activities include the processing of intermediate, bulk or finished phage products exposed to the environment, this must take place in an environment with specified air quality and cleanliness in order to minimize the risk of contamination. The effectiveness of these measures must be validated and monitored. Where intermediate, bulk or finished products are exposed to the environment during processing, without a subsequent microbial inactivation process, an *air quality* with particle counts and microbial colony counts equivalent to those of Grade A as defined in the current European Guide to Good Manufacturing Practice (GMP), Annex 1 and Directive 2003/94/EC is required with a background environment at least equivalent to GMP Grade D in terms of particles and microbial counts. The biosafety level (BSL) is determined by the host bacteria used in the production processes (e.g., BSL-2 for *Pseudomonas aeruginosa*).

C. Quality Assurance and Quality Control (QA/QC) specifications

Products/characteristics	Control test	Limits of acceptance	Recommended test procedures
C.1. Host bacteria used in production (stock suspensions)			
The bacterial hosts used in the production process – with the exception of selection, adaptation and efficiency of plating (EOP) and host range determination – should be as safe (or least pathogenic) as feasible.			
Origin	Document pedigree/ history/pathogenicity level	Known origin	Screening of scientific literature, lab books, consignment letters,..
Identification	Identification at the species and strain levels	Matching species and strain identification	• State of the art clinical microbiology techniques • Highly discriminating (molecular/ genomic) typing techniques (e.g., MLST, AFLP, PFGE, Rep-PCR,..)
Most often it will not be possible to find or quickly generate a suitable host bacterium that is free of prophages or phage-like elements, but one should nevertheless strive to use non-lysogenic strains, containing as few phages or other phage-like elements of genetic exchange [11, 12] as possible	• Induction of phages • Host genome screening for phage or phage-like elements	As few spontaneously produced (or by induction) temperate phages, complete prophage sequences or phage-like elements as possible[a]	• *In vitro* induction methods (Mitomycine C [13] or UV induction) • State of the art DNA sequencing and analysis (bioinformatics) procedures
Avoid mutator strains as host bacteria	Screen for mutator strains in case of doubt	No mutator strain	State of the art tests (e.g., fosfomycin and rifampicin Disk Diffusion Tests) [14]
Validated preservation/storage (cryopreservation, freeze-drying,..)	Monitor storage conditions (e.g., temperature)	Variable, depending on the preservation method	Variable (e.g., temperature probes, temperature indicator labels,..)
C.2. Bacteriophages (Master Seed lots)			
Origin		• Known origin	

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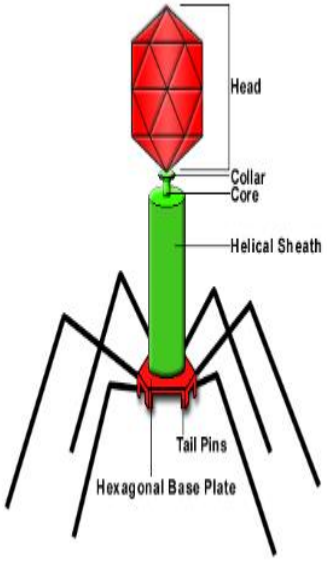
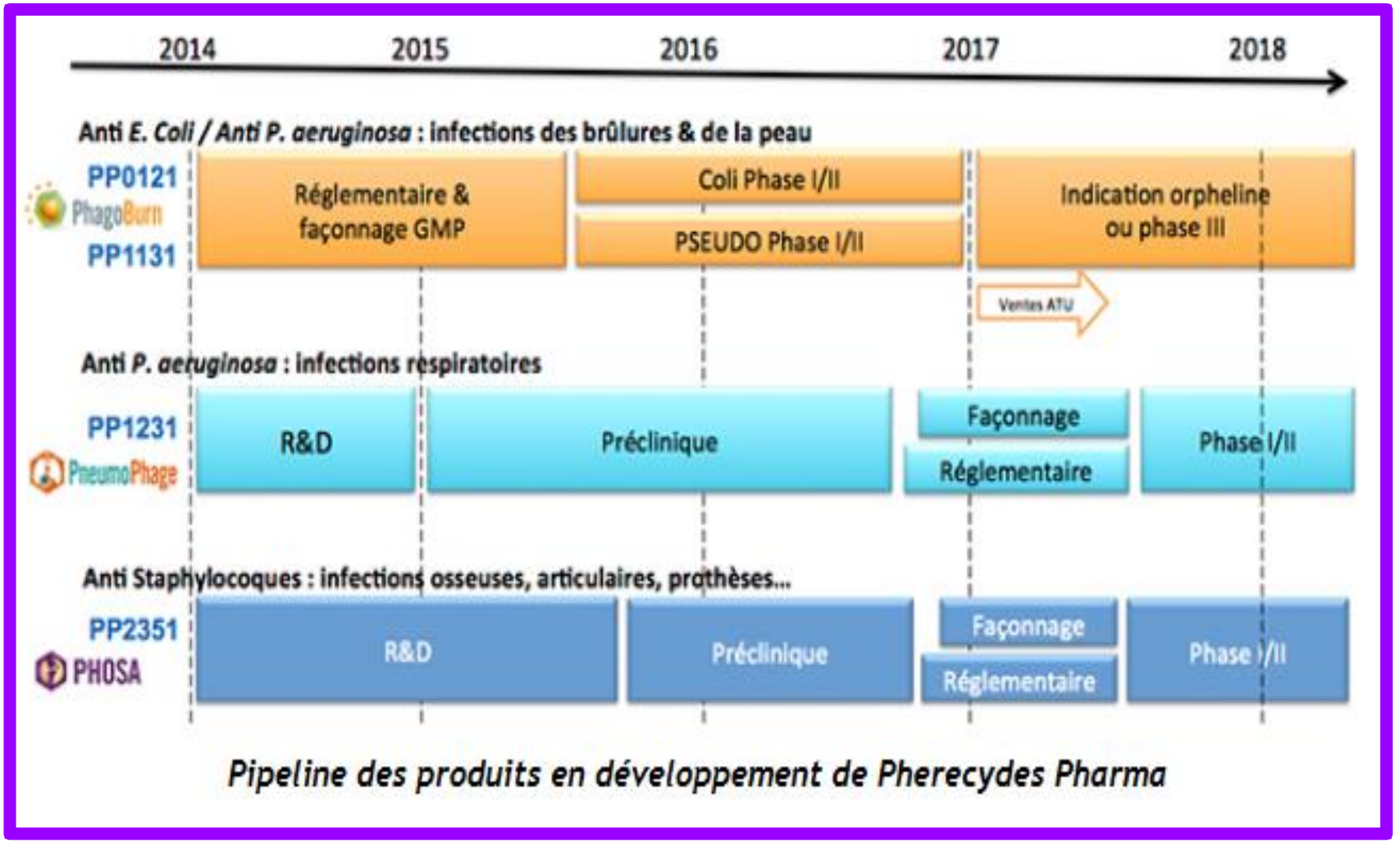
PHERECYDES PHARMA

GEEPhage Phagothérapie





Pipeline des produits en développement de Pherecydes Pharma

COMPANY	LOCATION	PRODUCTS	APPLICATIONS	IN TRIALS?
AmpliPhi	Richmond, Virginia	Natural phage cocktails	*P. aeruginosa* lung infections in cystic fibrosis; *S. aureus* wound and skin infections; *C. difficile* gastrointestinal infections	Phase 1 approved November 2015
ContraFect Corporation	Yonkers, New York	Bacteriophage lysins	*S. aureus* bacteremia	Phase 1 launched April 2015
EnBiotix	Cambridge, Massachusetts	Engineered phages	Staphylococcal infections of prosthetic joints	Preclinical
EpiBiome	San Francisco, California	Natural phage cocktails	*E. coli* and *Shigella dysenteriae* diarrheal infections in children	Preclinical
Fixed-Phage	Glasgow, U.K.	Natural phages fixed to solid surfaces	MRSA wound infections	Preclinical
Intralytix	Baltimore, Maryland	Natural phage cocktails	*S. aureus, P. aeruginosa, E. coli* wound infections; irritable bowel disease	Preclinical
Micreos	Wageningen, Netherlands	Bacteriophage lysins	*S. aureus* and MRSA skin infections	Preclinical
Novolytics	Warrington, U.K.	Natural phage cocktails	MRSA skin infections	Preclinical
Pherecydes	Romainville, France	Natural phage cocktails	*E. coli* and *P. aeruginosa* burn and skin infections; *P. aeruginosa* respiratory infections; *S. aureus* bone/joint/prosthetic infections	Phase 1 launched September 2015
Synthetic Genomics	San Diego, California	Engineered phages	Infections in burn wounds, skin, and cystic fibrosis	Preclinical
TechnoPhage	Lisbon, Portugal	Natural phage cocktails	Chronic ulcers, respiratory and skin infections	Preclinical

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GEEPhage
Phagothérapie

PROJETS STAPHYLOCOQUES ET OS

PHOSA

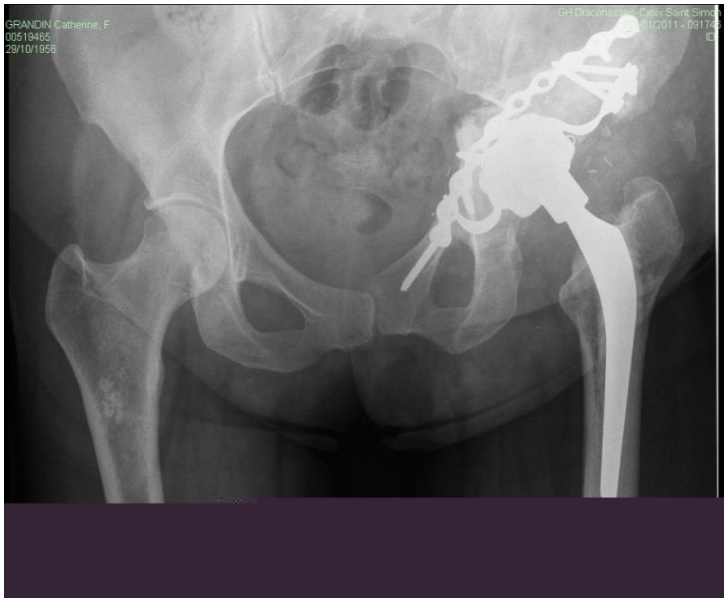
2015-2016

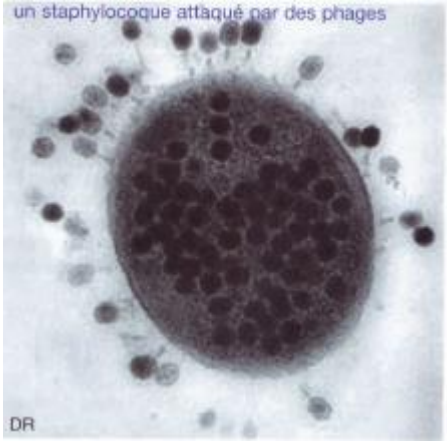
Cocktail de bactériophages pour lutter contre certaines infections bactériennes ostéo-articulaires provoquées par *Staphylococcus (aureus* et *epidermidis*)

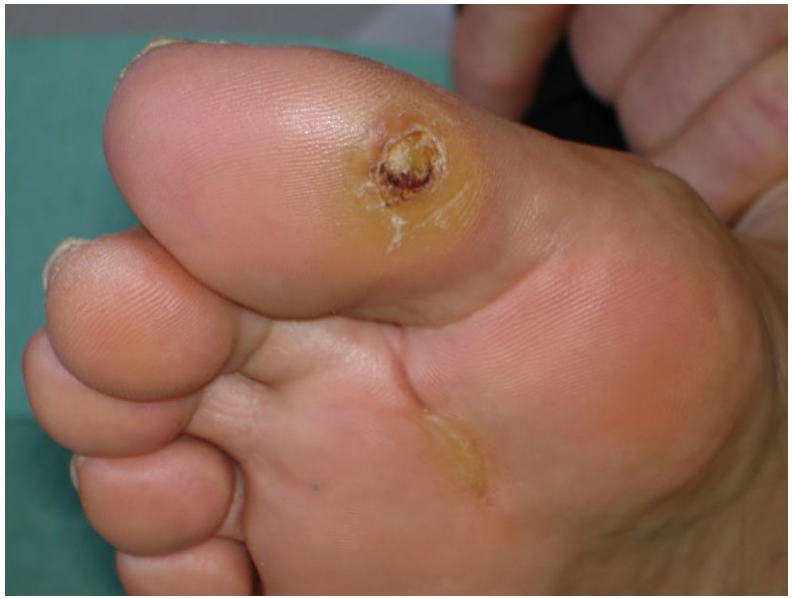
PHAGOS

2017?

PHAGOPIEDS







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Comparaison phage-antibiotique

GEEPhage
Phagothérapie

	Phagothérapie	***Antibiothérapie***
Mode d'action et pharmacologie	Un phage se multiplie dans le foyer infectieux, disparaît avec la bactérie. Dose unique théoriquement suffisante	Antibiotiques (ATB) métabolisés in vivo et diffusion variable Pharmacologie des ATB connue
Spécificité	Un phage s'attaque à une espèce bactérienne pathogène ciblée → respect des flores commensales	Un ATB à large spectre est actif sur plusieurs espèces bactériennes → non-respect des flores (diarrhée, mycoses)
Effets secondaires	Rares effets secondaires (fièvre, céphalée)	Nombreux effets toxiques (digestifs, allergiques, neurologiques, rénaux, cardiaques, tendineux, etc.)
Impact environnemental	Faible si les phages sont naturels Inconnu si phages génétiquement modifiés	Important si l'emploi massif (utilisation dans l'élevage)
Limites	Bactérie pathogène doit être isolée	Contre-indications connues (toxicité)
Résistance	R aux phages peut apparaître en cours de traitement → utiliser plusieurs phages (« cocktail ») Les phages restent actifs sur les bactéries R aux ATB et ne les sélectionnent pas	Les R aux ATB en augmentation pour toutes les espèces et pour tous les ATB partout dans le monde. Les R sont souvent multiples
Production et coût	Phage naturel peu coûteux, rapidement utilisable → intérêt pour les pays à faibles ressources Phage génétiquement modifié coûteux → brevet possible, délai, disponibilités ?	Mise sur le marché ATB longue et coûteuse → coût excessif pour les pays en voie de développement Désintérêt actuel de l'industrie pharmaceutique
Efficacité	Efficacité prouvée dans de nombreuses études exemples animales, études exemples humaines rares et limitées	Efficacité si les indications sont bien posées Échec si bactérie R ou non isolée Études exemples rigoureuses avec autorisation de mise sur le marché
Indications	Indications mal définies et n'existe aucune standardisation pour utilisation des phages	Prescription ATB standardisée, normes et indications bien établies (référentiels)
Réglementation	Phages, biomédicaments, absents des textes de la santé publique	Règlements bien adaptés pour fabrication et utilisation des ATB

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MODES D'ADMINISTRATION

GEEPhage Phagothérapie

Recent advances in bacteriophage therapy: how delivery routes, formulation, concentration and timing influence the success of phage therapy

JPP Journal of Pharmacy and Pharmacology

JPP 2011, ••: ••–••

Elizabeth M. Ryan, Sean P. Gorman, Ryan F. Donnelly and Brendan F. Gilmore

School of Pharmacy, Queens University Belfast, Belfast, UK

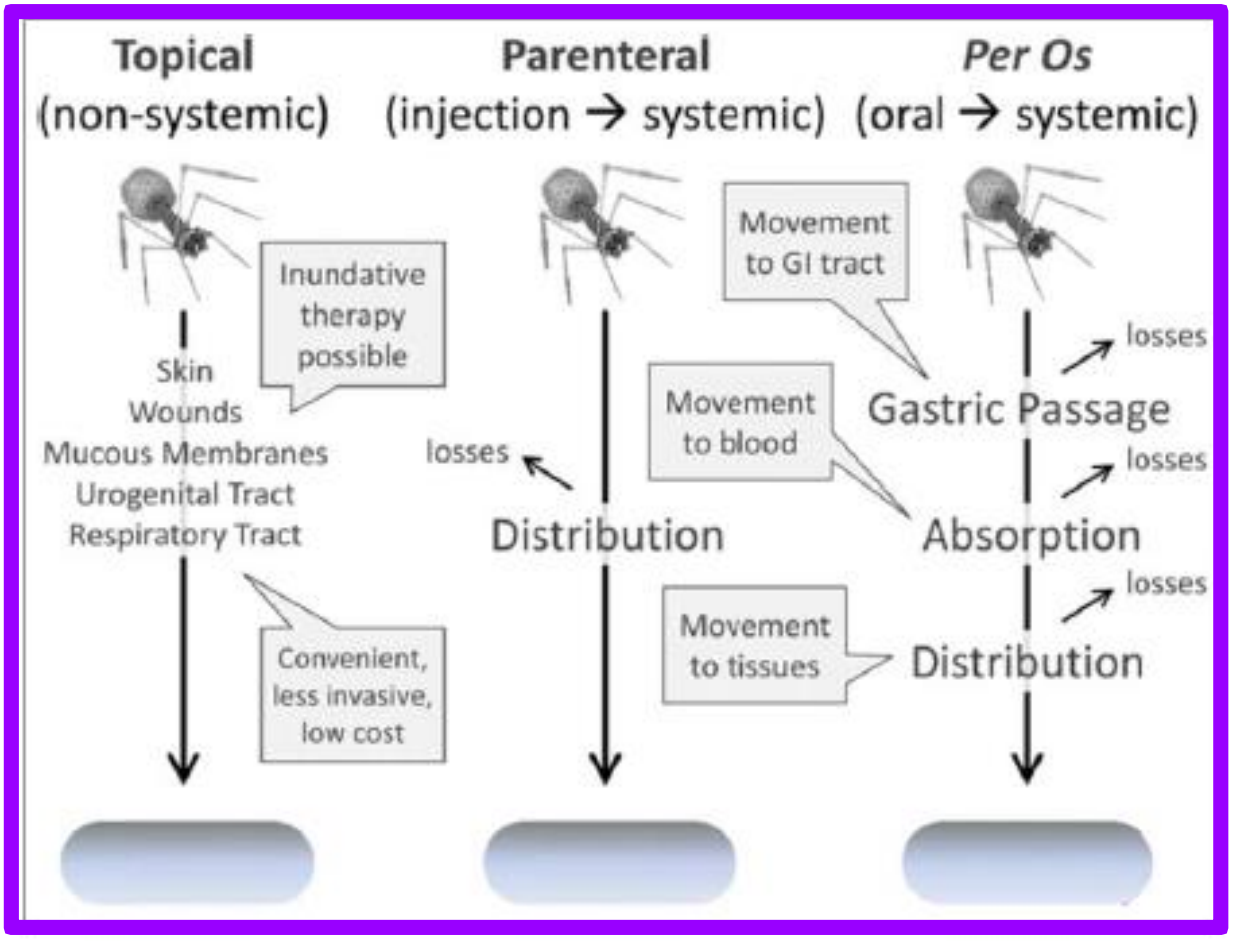
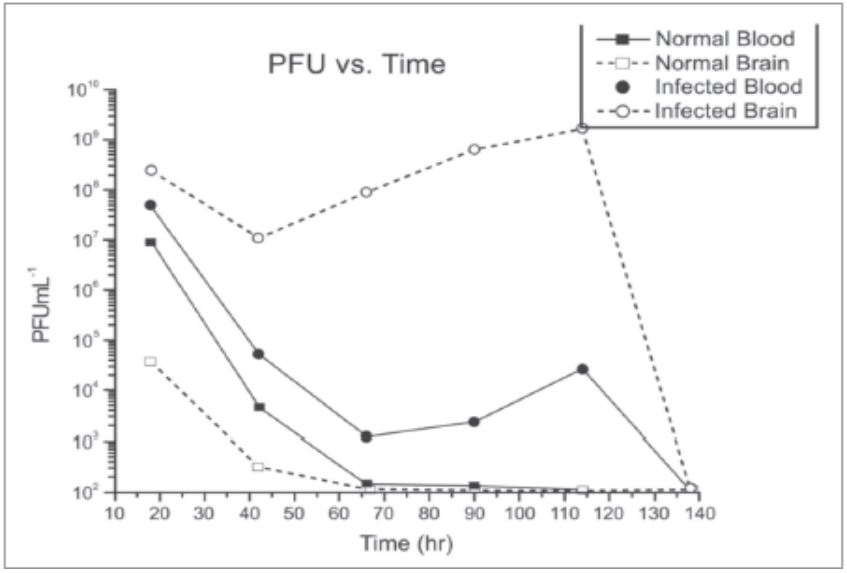


Figure 1. This figure, based on the data in the 1943 mouse studies of Rene Dubos,[78] provides significant insight into why phage therapy works well even in treating infections that antibiotics can't reach. When he injected the mice intraperitoneally with 10^9 phages, they quickly appeared in the blood stream, entering the brain, but they were rapidly cleared. However, if the mice were also injected intracerebrally with *Shigella dysenteriae*, the host for these phages, then 46/64 of the mice survived (as compared with 3/84 in the absence of appropriate viable phage) and the brain level of phage climbed to over 10^9 per gram. Once the bacteria were cleared, phage levels dropped below detection limits.



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frontiers in Microbiology

Means to Facilitate the Overcoming of Gastric Juice Barrier by a Therapeutic Staphylococcal Bacteriophage A5/80

Ryszard Międzybrodzki[1,2,3], Marlena Kłak[1,4], Ewa Jończyk-Matysiak[1], Barbara Bubak[1], Anna Wójcik[1], Marta Kaszowska[5], Beata Weber-Dąbrowska[1], Małgorzata Łobocka[6,7] and Andrzej Górski[1,2,3]*

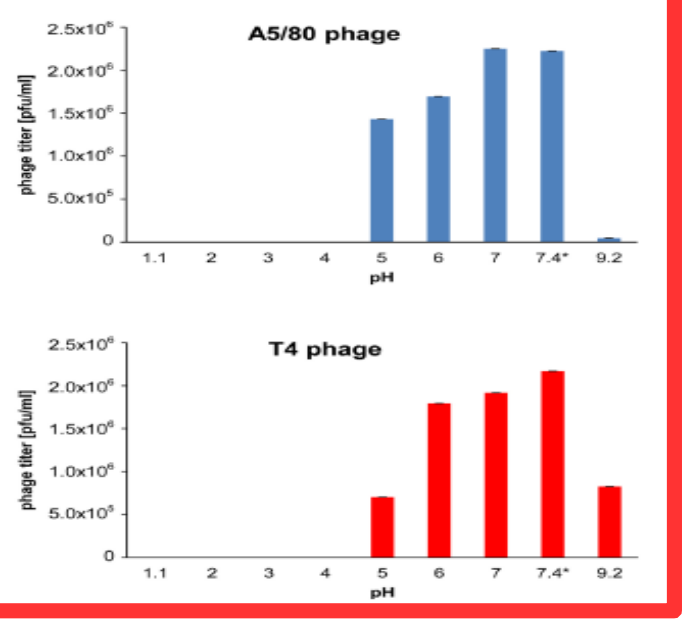

FIGURE 1 | Effect of acidity on phage survival. *Phosphate buffered saline (pH 7.4) was used as control. The phages were incubated in buffers at 37°C for 60 min.

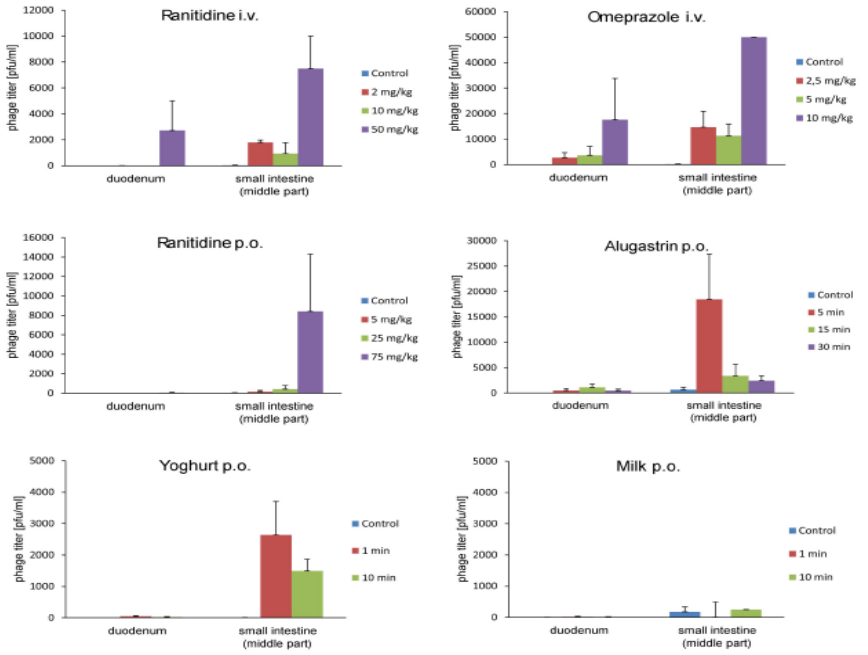

FIGURE 2 | Influence of ranitidine, omeprazole, Alugastrin, yogurt, and milk on A5/80 phage ability to survive in the stomach and to pass into the small intestine. Samples for phage titer determination were taken 30 min after oral administration of 0.5 ml of phage lysate (10^7 pfu/ml). Groups of animals differed

TABLE 1 | Comparison of the bioavailability of A5/80 phage at different time points after its oral versus intravenous administration to rats.

Time since the phage administration	Phage titer after oral administration [pfu/ml]				Phage titer after intravenous administration [pfu/ml]			
	Blood		Liver		Blood		Liver	
	n	Mean	*n*	Mean	*n*	Mean	*n*	Mean
1 h	2	0	2	0	3	485†	2	419‡
2 h	3	0	2	0	1	50	–	–
4 h	2	0	2	0	1	150	1	33
18 h	2	0	2	0	1	5	1	5

All animals received phage at dose of 2 × 10^9 pfu (in 1.0 ml of phage lysate). Alugastrin (1.0 ml) was applied 15 min before the phage administration. †, min.-max. value: 405–555 pfu. ‡, min.-max. value: 373–465 pfu. No phage was detected neither in blood nor liver in a control group of two animals (non-treated with the phage). N, number of tested animals.

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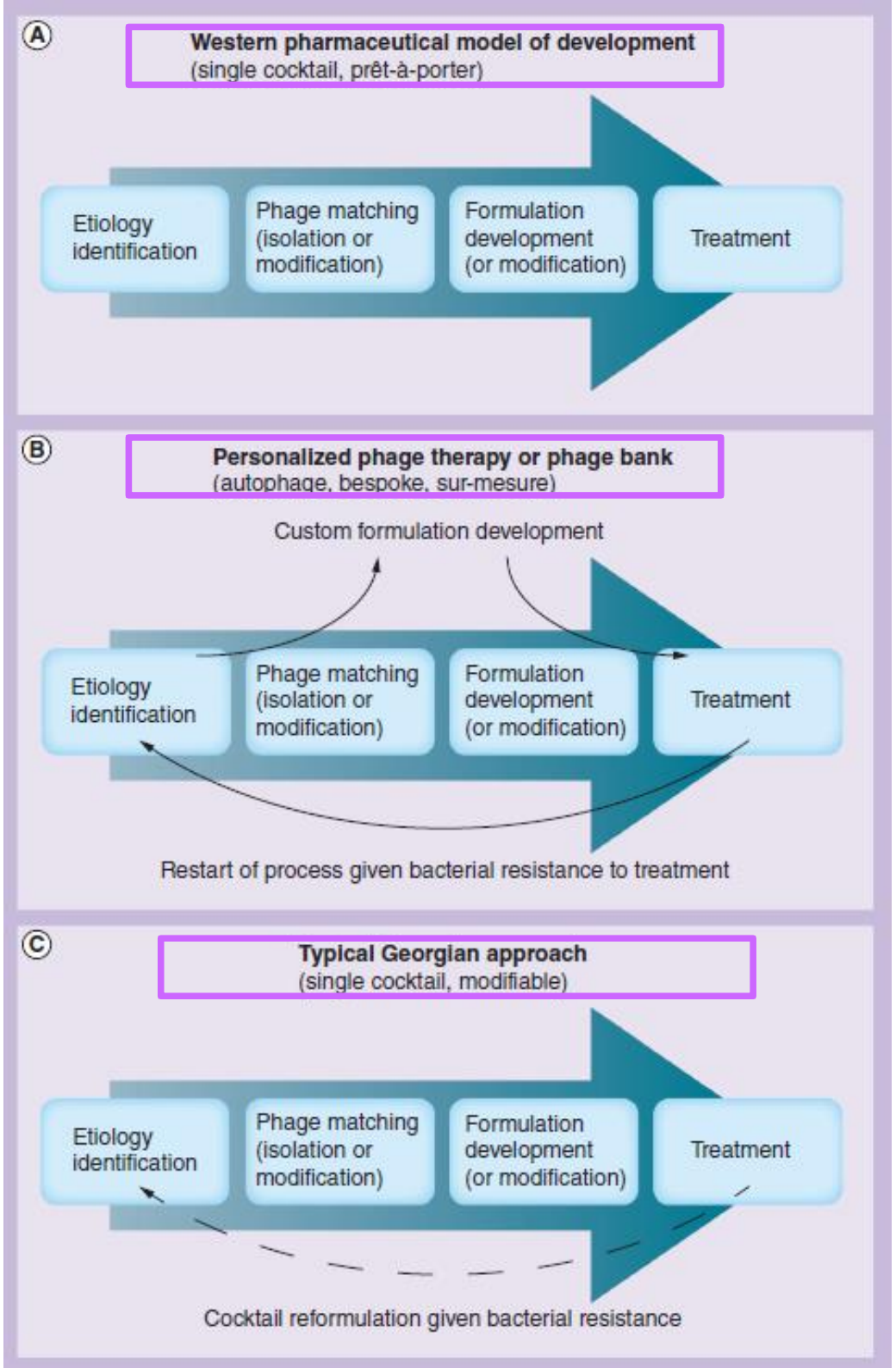

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Table 2. Models of formulation for antibacterial treatment.

Type of formulation	Approach	Antibacterial types used per formulation	Personalized medicine	Prior to use characterization of infection[†]	Breadth of spectrum of activity	Flexibility (to bacterial resistance)[‡]	Flexibility (in spectrum of activity)[§]
Phage bank (monophage)	Sur-mesure	1	+++	+++	+	+++	+++
Personalized cocktail	Sur-mesure	>1	+++	+++	++	+++	+++
Cocktail bank	Sur-mesure via prêt-à-porter	>1	++	++	+++	++	+
Single cocktail	Prêt-à-porter	>1 or >>1	–	+	+++	–[¶]	–
Typical antibiotic	Prêt-à-porter	1	–	+	++++	–	–
Narrow-spectrum antibacterial[#]	Prêt-à-porter	1	+	≥+	≥+	–	–
Single cocktail	Prêt-à-porter but modifiable	>1 or >>1	+	+	+++	+[††]	++

[†]Degree to which etiologies must be identified to achieve reasonable likelihood of treatment success.
[‡]Potential to respond, in a clinic, to treatment failures resulting from infection resistance to a given phage formulation.
[§]Potential for modification of formulation used for treatment over subyear time scales.
[¶]Should phages prove able to adapt in vivo to resistant bacteria, then this designation may be modified towards increased flexibility; see [28] for discussion of this potential.
[#]Such as monoclonal antibodies [100], enzymes [101] or bacteriocins [102]; proposed characteristics assume that the antibacterial diversity of such agents is small relative to that of phages.
[††]This designation refers to an inability to respond to resistance in specific patients when using a fixed cocktail, but nonetheless that such patients may be subsequently treated given sufficiently rapid updating of otherwise modifiable cocktail formulations by manufacturers.

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PHAGOTHERAPIE 2020

Modular Approach to Select Bacteriophages Targeting *Pseudomonas aeruginosa* for Their Application to Children Suffering With Cystic Fibrosis

Victor Krylov[1], Olga Shaburova[1], Elena Pleteneva[1], Maria Bourkaltseva[1], Sergey Krylov[1], Alla Kaplan[1], Elena Chesnokova[1], Leonid Kulakov[2], Damian Magill[2] and Olga Polygach[1]*

[1] *Laboratory for Genetics of Bacteriophages, Department of Microbiology, I.I. Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia,* [2] *Medical Biology Centre, School of Biological Sciences, Queen's University Belfast, Belfast, UK*

for the growth of bacteria of various species. At the early stages, staphylococcal infection is the major culprit, followed by *P. aeruginosa* domination (Kosorok et al., 2001). The production of alginate by these bacteria, an extremely viscous polysaccharide, by these bacteria promotes the proliferation and survival of other hazardous species, such as *Burkholderia sp.*, worsening the overall prognosis (Henry et al., 1992; Lynch and Dennis,

During the course of infection a gradual change in the properties of the primary infecting strains of *P. aeruginosa* takes place, manifested by a decrease in their pathogenicity and virulence, as well as increased sensitivity to the lytic effect of bacteriophages (Friman et al., 2013; Cullen et al., 2015). Moreover these strains, being adapted to the conditions of the lungs, are influencing the expression of the pathogenic properties of other species in the concomitant microflora (such as *Burkholderia multivorans*, *Burkholderia cenocepacia*, *Pandoraea pulmonicola* and *Pandoraea apista*) significantly lowering them.

FIGURE 1 | Official permission for application of phage therapy in CF unit of The Central Republican Children Clinical Hospital, Moscow, Russia and agreement of physicians in CF unit to collaborate in the study (for request of grant support).

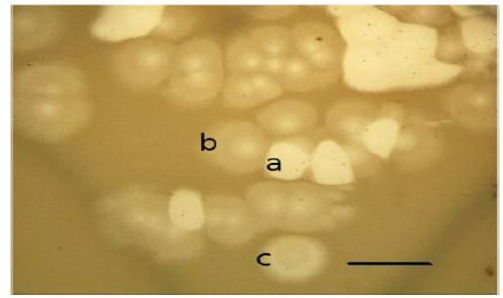

FIGURE 8 | Diversity of bacteriophage phiCHU plaques on lawn *P. aeruginosa* PAO1 (pMG53). The replanting of phages from clear **(a)**, turbid **(b)** or semiclear **(c)** plaques again leads to production of all types of plaques. Bar is 1 cm.

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GEEPhage
Phagothérapie

NECESSITE DE CREATION D'UNE BANQUE DE BACTERIOPHAGES

DSMZ

P-H-A-G-E.org

Phages for Human Applications Group Europe vzw
Militair Hospitaal Koningin Astrid
C DIS/Site NOH, Blok C, 1ste verdieping
Lokaal 1.391
Bruynstraat 1
1120 BRUSSEL

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EN ATTENDANT: Où TRAITER?

En Géorgie?

SANTÉ Serge Fortuna a frôlé l'amputation, depuis il milite pour que la phagothérapie soit autorisée

Sa jambe sauvée en Géorgie par des virus guérisseurs

VEDÈNE

Il y a un peu plus de l'amputation. Très près. Il en avait d'ailleurs lui-même fait la demande à un médecin à Nîmes. Serge Fortuna est retombé sur un documentaire qui a changé sa vie. Sur Arte, l'émission parle de ce docteur Alain Dublanchet, microbiologiste à l'hôpital de Villeneuve-Saint-Georges. Celui-ci aspire en Géorgie chercher des virus bactériophages pour sauver la jambe d'une de ses patientes. Serge Fortuna y voit la dernière chance pour sauver la sienne. En novembre dernier, il débarque à Tbilisi, la capitale géorgienne.

Ce staphylocoque doré, il l'a attrapé il y a un accident de motocross. Fractures ouvertes, greffes, opérations, hospitalisation, puis infection. « Sauf qu'à l'époque on ne parlait pas d'infection nosocomiales, raconte Serge Fortuna, on m'a dit que j'avais la gangrène ».

« J'ai demandé l'amputation, lettre de motivation à l'appui »

La bactérie mole quinze semaines et un vaisseau je lonnée de séjours à l'hôpital. Avec 39 opérations au total, il n'a pas peur de parler de « succession de mutilations ». « J'avais baissé les bras, j'ai réclamé l'amputation, lettre de motivation à l'appui ».

C'est dans la que les phages sont entrés dans sa vie.

Serge Fortuna prépare son prochain voyage, en Pologne cette fois où la phagothérapie est autorisée. « Je veux voir si l'on peut faire des passerelles avec la France, si l'on peut envisager que ce soit remboursé par la Sécurité ». Photo Richard WATRIN

Cette thérapie, tombée dans l'oubli en France, est toujours utilisée en Europe de l'Est. La technique est simple : introduire des virus mangeurs de bactéries pour éliminer l'infection (voir ci-dessus). Alors que le corps humain est de plus en plus résistant aux antibiotiques, la phagothérapie, méthode quasiment naturelle et sans danger, pourrait selon le professeur Alain Dublanchet, sauver des membres de l'amputation et même des vies.

En Géorgie, Serge Fortuna débarque avec juste une adresse, et le Chaîne du routard. La clinique qu'il a trouvée sur internet n'est pas vraiment engageante. « Je ne savais pas où j'allais, il n'y avait même pas de plaque à l'entrée, mais je n'avais pas le choix ». Des amoules à boire, des pansements sur la jambe… les phages ont fait le boulot, en 20 jours seulement.

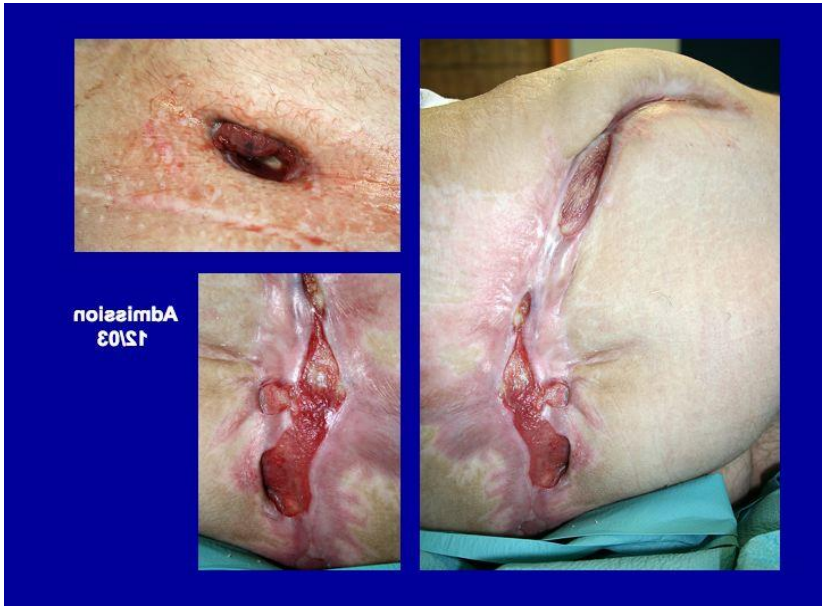
Guéri grâce à un traitement à 6 € la boîte

« Là-bas, on trouve le traitement sans ordonnance et pour six euros la boîte ». Son parcours médical a coûté des centaines de milliers d'euros à la Sécurité sociale française. En Géorgie, il a dépensé au total 30 euros. Les résultats de ses analyses sont terribles. Il y a quatre jours et plus aucune trace de la bactérie. Mêmes résultats pour le Dr Dublanchet, il faut trois à quatre ans de recul pour soigner la guérison d'une cavité complète.

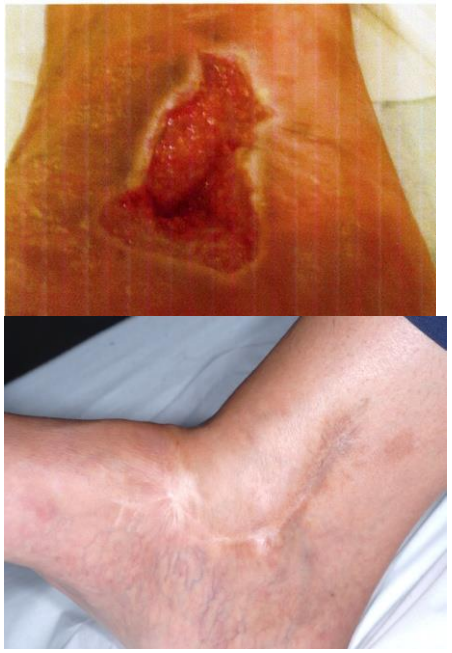
Serge Fortuna, réalisateur dernier, a donc décidé de faire de son espérance, le combat de sa vie. Un film en préparation (voir dessous), un livre en cours, des conférences… Il veut médiatiser le plus possible cette technique médicale.

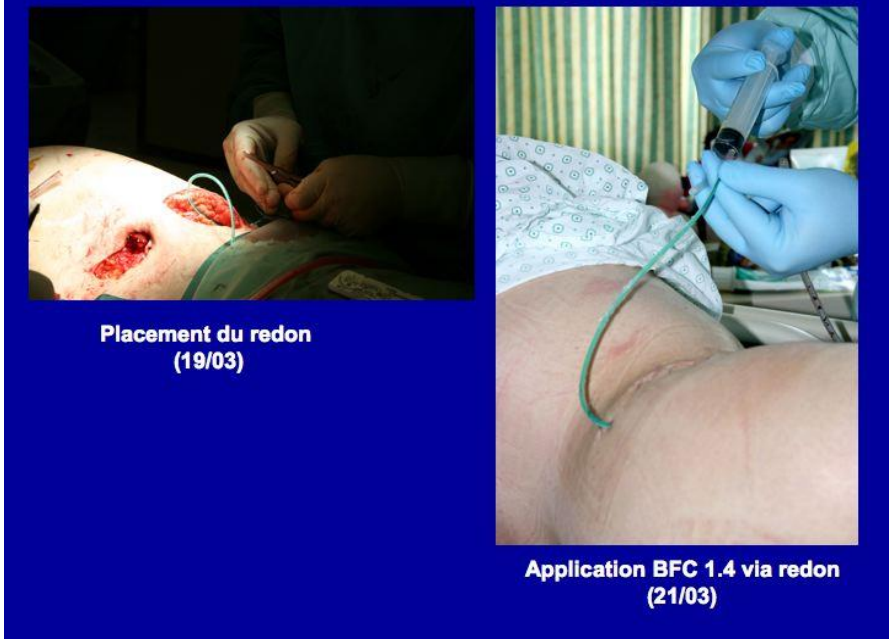
En attendant que change un jour la législation sur la phagothérapie, il se bat pour les autres malades. « Il faut que les gens qui ont en besoin sachent qu'il existe cette alternative. Je veux leur dire qu'il n'y a plus rien à craindre à y aller, qu'il faut y aller ».

À 56 ans, Serge Fortuna va décontinuer pour rentrer ses documentaires, ses voyages, une vie normale. « Mais je resterai à l'affût sur le sujet… jusqu'à la fin de ma vie »



Dans l'Union Européenne?





Clinical Aspects of Phage Therapy

Ryszard Międzybrodzki,[*,†,1] **Jan Borysowski,**[‡] **Beata Weber-Dąbrowska,**[*,†] **Wojciech Fortuna,**[*,†] **Sławomir Letkiewicz,**[†,§] **Krzysztof Szufnarowski,**[†,‖] **Zdzisław Pawełczyk,**[†] **Paweł Rogóż,**[†,¶] **Marlena Kłak,**[*] **Elżbieta Wojtasik,**[#] and **Andrzej Górski**[*,†,‡]

† Phage Therapy Unit, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

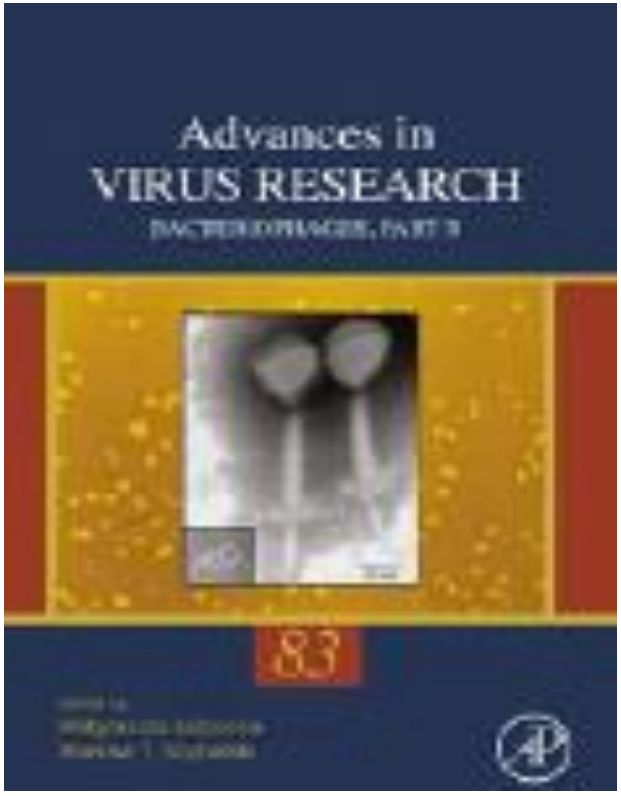


TABLE II Status of bacteriophage collection of the Institute of Immunology and Experimental Therapy (May 2011)

Bacteriophage host	Number of phages
Escherichia coli	121
Klebsiella pneumoniae or *Klebsiella oxytoca*	95
Enterococcus faecalis or *Enterococcus faecium*	73
Enterobacter cloacae	48
Shigella flexneri or Shigella sonnei	39
Citrobacter freundii	38
Pseudomonas aeruginosa or *Pseudomonas fluorescens*	37
Salmonella enteritidis or *Salmonella typhimurium*	32
Stenotrophomonas maltophilia	18
Serratia marcescens or *Serratia liquefaciens*	17
Proteus mirabilis	17
Morganella morganii	14
Staphylococcus aureus	7
Acinetobacter baumannii	5
Burkholderia cepacia	2


Centrum Medyczne
Instytutu Immunologii i Terapii Doświadczalnej PAN
Ośrodek
Terapii Fagowej
Phage Therapy
Unit


Centrum Medyczne Instytutu Immunologii i Terapii Doświadczalnej PAN
Ośrodek Terapii Fagowej
Phage Therapy Unit

TABLE III Characteristics of the 157 patients admitted for phage therapy at the Phage Therapy Unit in 2008–2010

Gender	Males: $n = 89$ Females: $n = 68$
Median age	Males: 44.5 (21–79) years Females: 47 (20–82) years Total: 46 (20–82) years
Diagnosis	Genital and urinary tract infection in men: chronic bacterial prostatitis ($n = 14$); urinary infection ($n = 10$); chronic bacterial prostatitis/urinary infection ($n = 6$) Genital and urinary tract infection in women: urinary infection ($n = 14$); vaginal infection ($n = 3$); urinary/vaginal infection ($n = 5$) Soft tissue infection: postoperative wound infection ($n = 6$); leg ulcer ($n = 8$); abscess, phlegmon, or empyema penetrating to the body cavities ($n = 5$); deep tissue infection ($n = 11$) Skin infection: external ear infection ($n = 4$); unspecified local infection of skin ($n = 3$); atopic dermatitis complicated by staphylococcal infection ($n = 1$); acne ($n = 1$); eczema ($n = 1$); furunculosis ($n = 1$) Orthopedic infections: prosthetic joint infection ($n = 8$); osteomyelitis ($n = 22$); joint infection ($n = 5$); osteomyelitis/joint infection ($n = 2$); discitis ($n = 1$) Respiratory tract infections: upper respiratory tract infections ($n = 17$); lower respiratory tract infections ($n = 4$); upper and lower respiratory tract infections ($n = 3$) Other: internal ear infection ($n = 1$), recurrent bacteremia ($n = 1$)
Pathogens causing the infection	Monoinfections: *S. aureus* ($n = 76$, including seven MRSA cases); *S. haemolyticus* ($n = 1$); *E. faecalis* ($n = 17$); *E. coli* ($n = 15$); *P. aeruginosa* ($n = 13$); *P. putida* ($n = 1$); *E. cloacae* ($n = 1$); *K. pneumoniae* ($n = 1$); *Salmonella* group C ($n = 1$) Polyinfections: *S. aureus* (MRSA)/*S. mitis* ($n = 1$); *S. aureus*/*A. junii* ($n = 1$); *S. aureus*/*E. cloacae* ($n = 1$); *S. aureus*/*M. morganii* ($n = 1$); *S. aureus*/*P. aeruginosa* ($n = 1$); *E. faecalis*/*K. oxytoca* ($n = 1$); *E. faecalis*/*C. freundii* ($n = 1$); *E. faecalis*/*E. coli*/*K. oxytoca* ($n = 1$); *E. faecalis* /*P. aeruginosa* ($n = 1$); *E. faecalis*/*P. vulgaris* ($n = 1$); *E. faecalis*/*S. aureus* ($n = 1$); *E. faecalis*/coagulase negative staphylococcus ($n = 1$); *E. coli*/*E. faecalis* ($n = 9$); *E. coli* – two different strains ($n = 1$); *E. coli*/*K. pneumoniae* ($n = 1$); *P. aeruginosa*/*S. aureus* ($n = 1$); *P. aeruginosa*/*S. aureus*/*P. vulgaris* ($n = 1$); *P. aeruginosa*/*S. maltophilia* ($n = 1$); *P. aeruginosa* /group F β-hemolytic *Streptococcus* ($n = 1$); *P. aeruginosa*/*A. baumannii* ($n = 1$); *P. vulgaris*/*E. coli*/*E. faecalis* ($n = 1$)
Concomitant antibacterial treatment	Antibiotics/chemotherapeutics: $n = 31$ Antibiotics/chemotherapeutics and disinfectants: $n = 6$ Antibiotics/chemotherapeutics and herbs or supplements for treatment of urinary infections: $n = 4$ Disinfectants: $n = 3$
Number of patients included in analysis	Patients for whom information on the treatment effect was available: $n = 153$ Patients for whom at least one laboratory result during PT was available: $n = 154$ Patients for whom complete information on cumulative treatment duration was available: $n = 49$

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Category of response to treatment	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)	
	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**

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« TOURISME MEDICAL »

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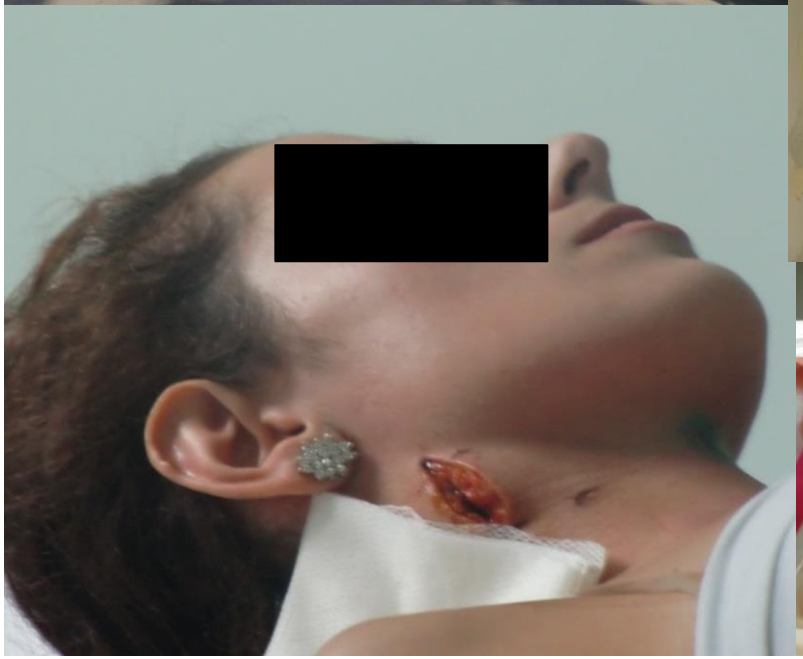

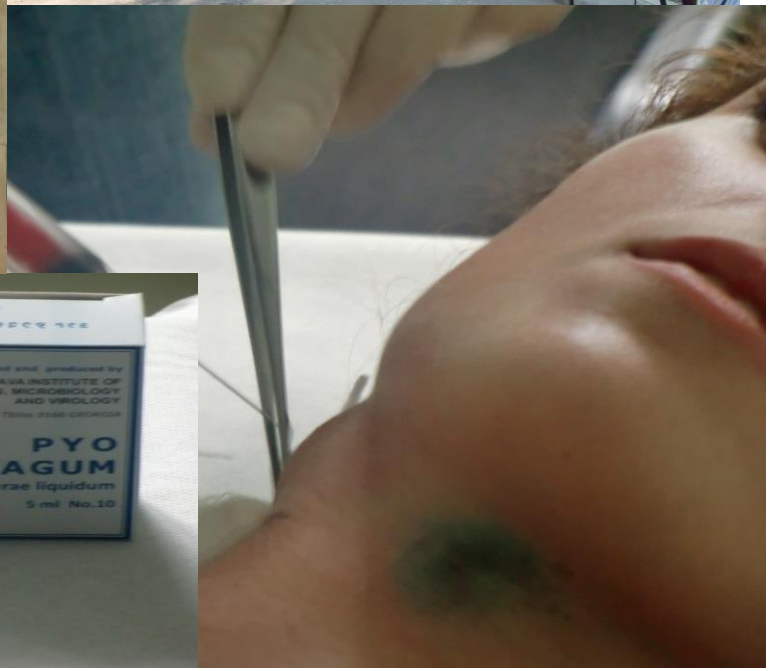

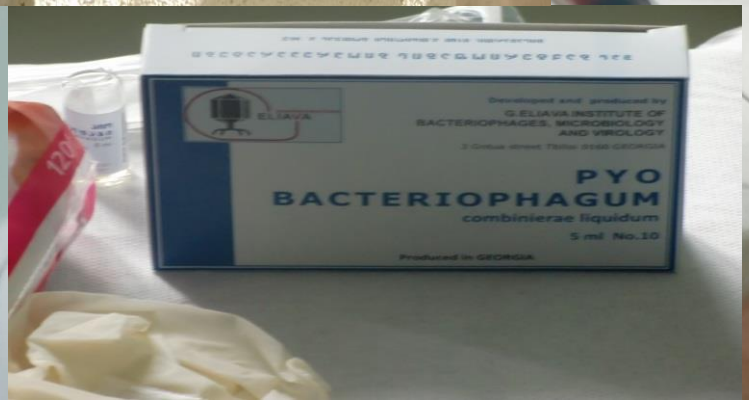

SE SOIGNER EN GEORGIE

69 Sanavardo Street 0104 Tbilissi Georgia

Alain LAVIT

Tél : 00995 322 981 995

Mail : contact@sesoignerengeorgie.com

Sommaire

Quantités	Prestations	Prix en €/UNITE	Prix en €/TOTAL
2	**Vols Paris 14h15 Tbilissi 02h55-Bagages inclus**	240,77 €	481,54 €
1	**Transfert Aller- Aéroport**	0 €	0 €
1	**15 Transferts Appartement-Institut Eliava**	200 €	200 €
21	**Jours Appartement à Tbilissi**	48 €	1008 €
1	**Analyse Institut Eliava**	120 €	120 €
15	**Accompagnateur francophone pour 15 jours**	28 e	420 €
1	**Traitement Phagothérapie 3 semaines à L'Institut Eliava**	3630 €	3630€
1	**Transfert Appartement - aéroport pour 2 personnes**	0 €	0
2	**Vols retour Tbilissi 06h20 - Paris 13h25 Bagages inclus**	0,00 €	0,00 €
Option	**Massage bains turcs privatisés-Transferts inclus**	50€	0
Option	**Journée de visite de Tbilissi avec guide + transferts + restaurant**	96€	0
Option	**Dîners chez l'habitant (transferts inclus)**	30€	
		Prix total	**5 859,54€**

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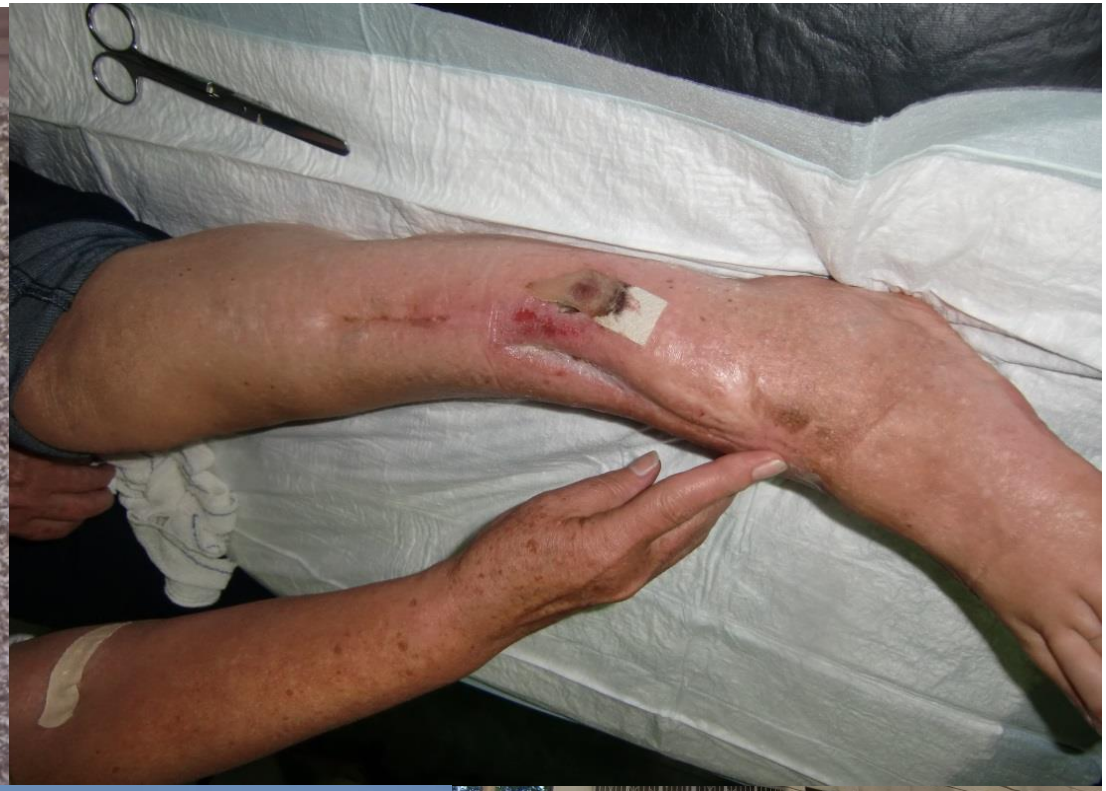

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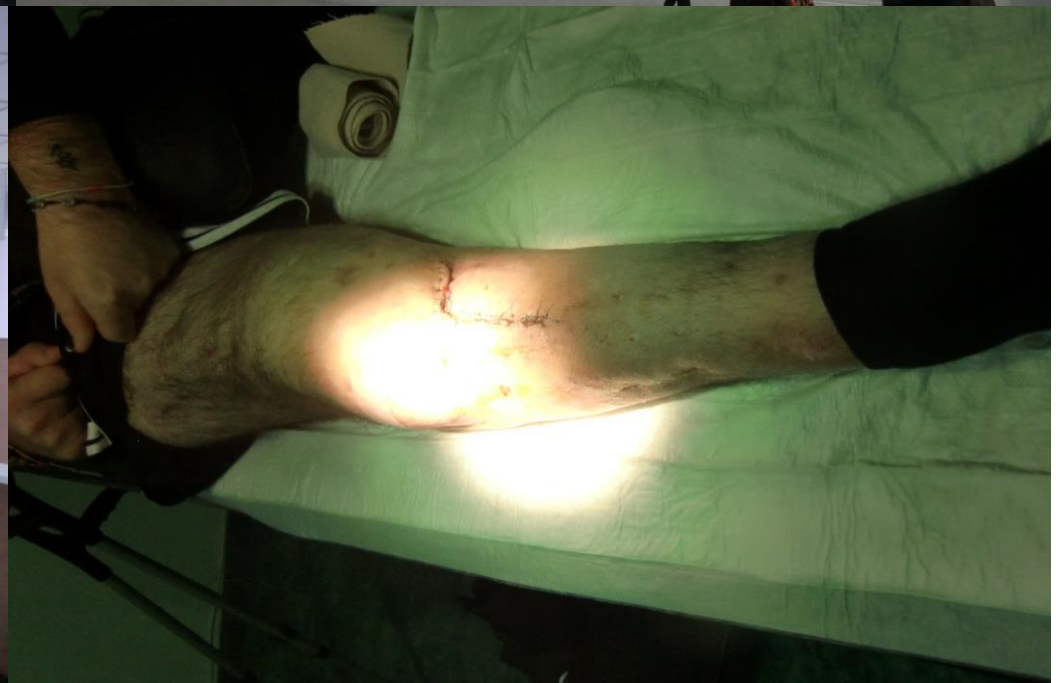

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PLACE DE L'ETHIQUE

Développement d'un tourisme médicale sans prise en charge financière:

- coût du transport
- coût du logement et séjour
- coût des traductions
- coût des traitements médicaux, voir chirurgicaux
- sécurité sanitaire (BMR, BK XDR)

Nécessité d'une mobilité suffisante, d'un accompagnement..

Éloignement familial et social prolongé

⟶ **INEGALITE DEVANT LA MALADIE**

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Declaration d'Helsinki de L'AMM - Principes éthiques applicables à la recherche médicale impliquant des êtres humains

Interventions non avérées dans la pratique clinique

37. Dans le cadre du traitement d'un patient, faute d'interventions avérées ou faute d'efficacité de ces interventions, le médecin, après avoir sollicité les conseils d'experts et avec le consentement éclairé du patient ou de son représentant légal, peut recourir à une intervention non avérée si, selon son appréciation professionnelle, elle offre une chance de sauver la vie, rétablir la santé ou alléger les souffrances du patient. Cette intervention devrait par la suite faire l'objet d'une recherche pour en évaluer la sécurité et l'efficacité. Dans tous les cas, les nouvelles informations doivent être enregistrées et, le cas échéant, rendues publiques.

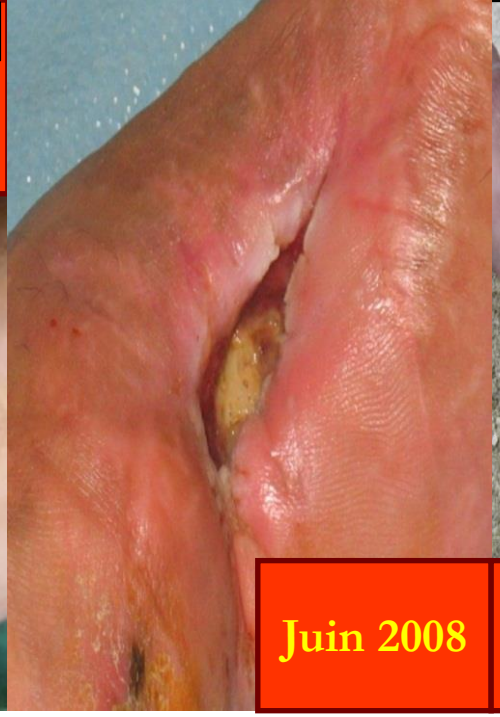
QUE DIT LE COMITE NATIONAL D'ETHIQUE ? ….. RIEN

IMPASSES THERAPEUTIQUES
COOPERATION ANTIBACTERIENNE

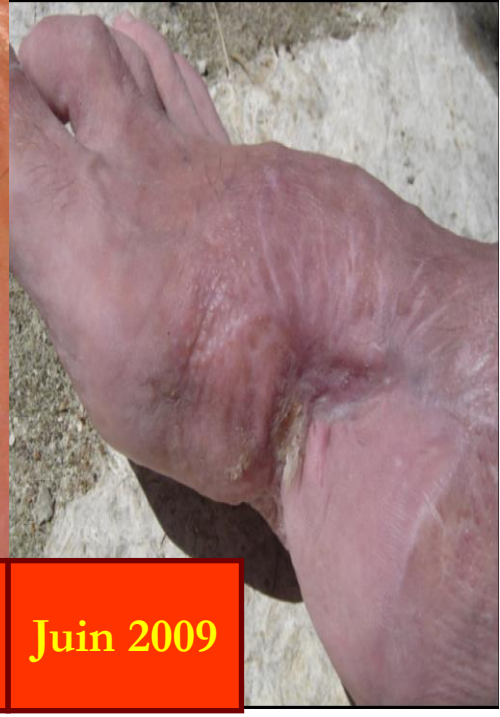
* Action lytique rapide exponentielle des phages
* Action lytique curative et préventive sur les biofilms permettant la libération planctonique des bactéries
* Suppression de l'effet délétère des biofilms (notamment sur l'apoptose des ostéoblastes)
* Effet synergique avec les antibiotiques

Cas 1: Homme de 45 ans [redacted]
Polytraumatisme le 10 février 2005 (37 fractures)
avec ostéite chronique du pied à SARM à J30

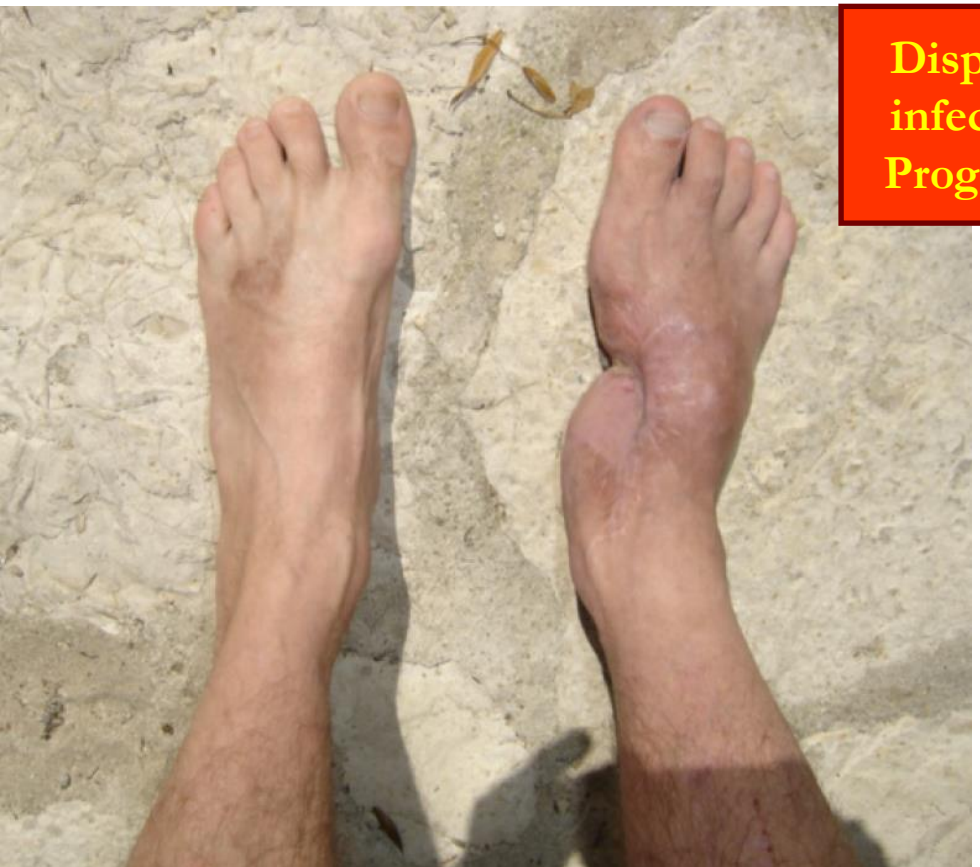
Intervention avec Phagothérapie en mars 2008



Juin 2008

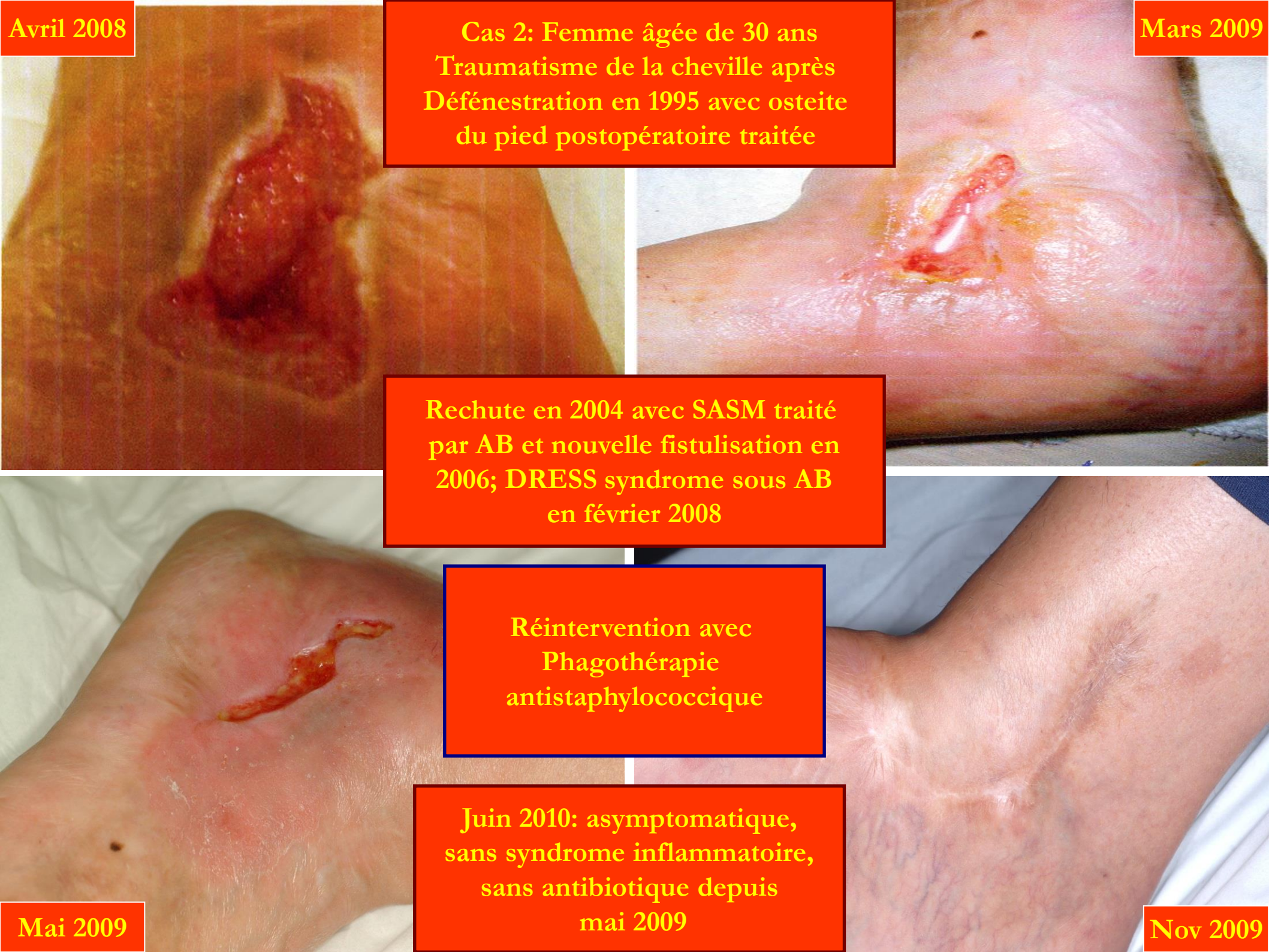


Juin 2009



Disparition des signes infectieux et fermeture Progressive de la plaie

Eté 2009


Avril 2008
Cas 2: Femme âgée de 30 ans
Traumatisme de la cheville après
Défénestration en 1995 avec osteite
du pied postopératoire traitée
Mars 2009
Rechute en 2004 avec SASM traité
par AB et nouvelle fistulisation en
2006; DRESS syndrome sous AB
en février 2008
Réintervention avec
Phagothérapie
antistaphylococcique
Juin 2010: asymptomatique,
sans syndrome inflammatoire,
sans antibiotique depuis
mai 2009
Mai 2009
Nov 2009

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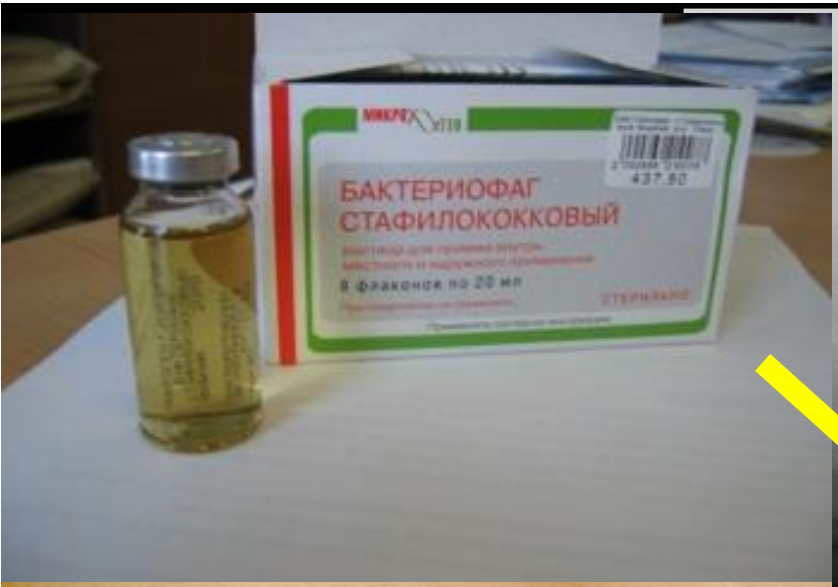

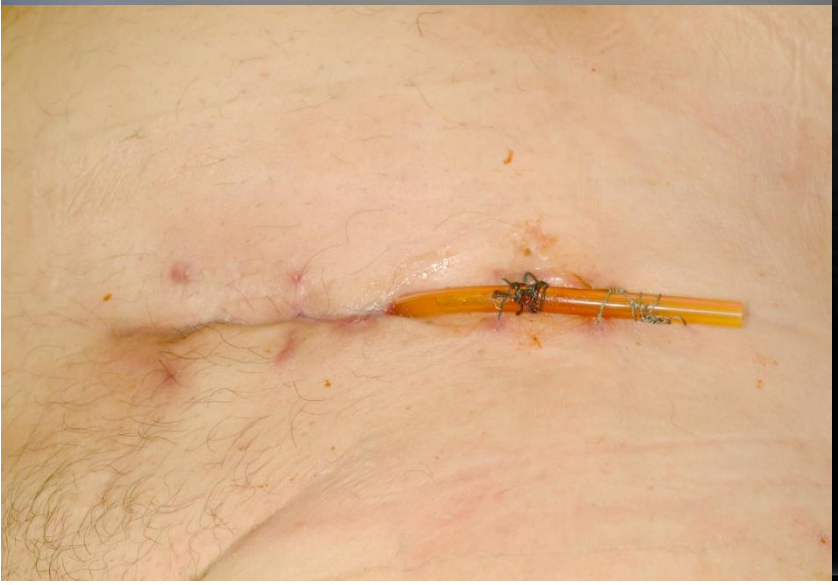

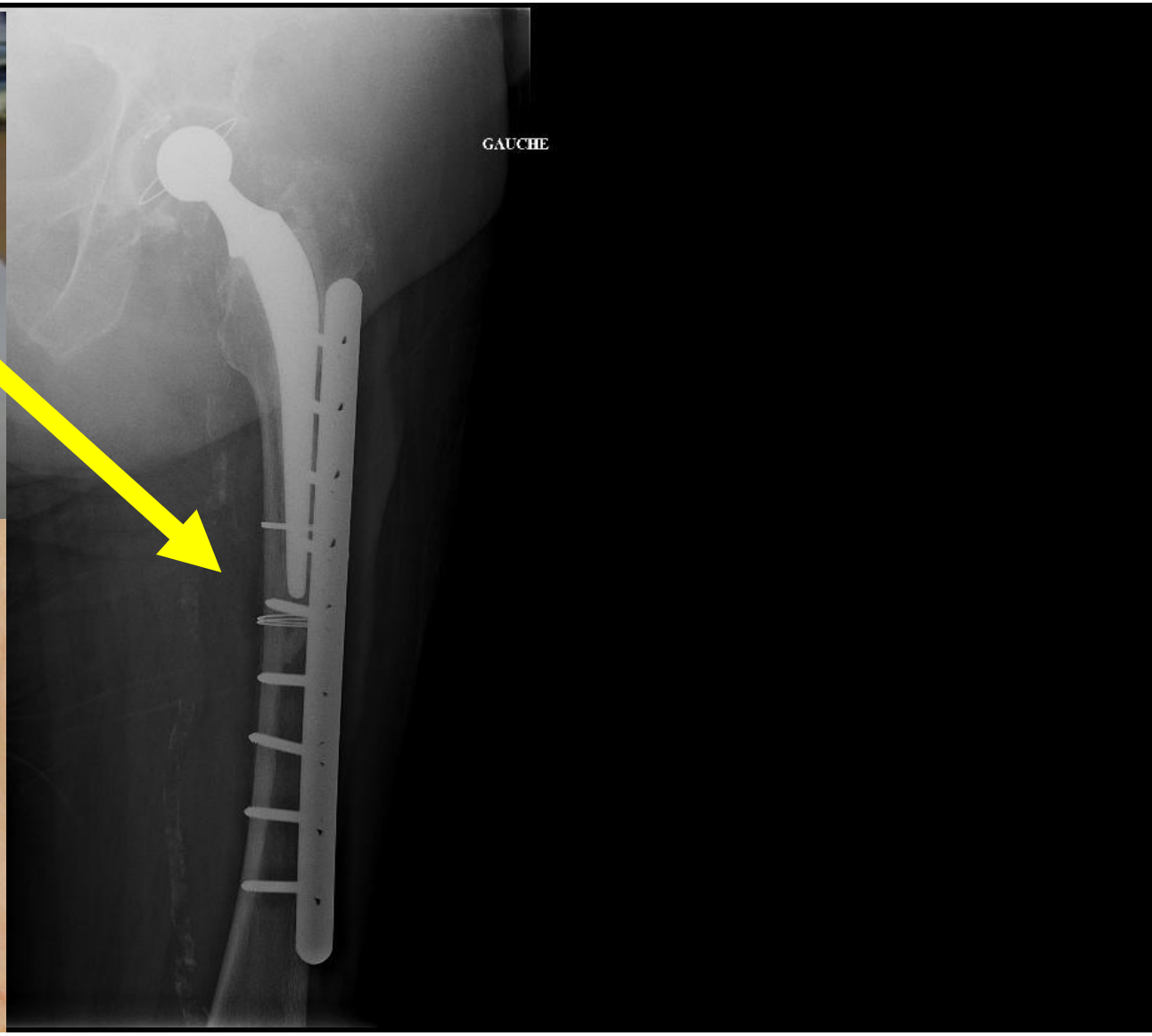

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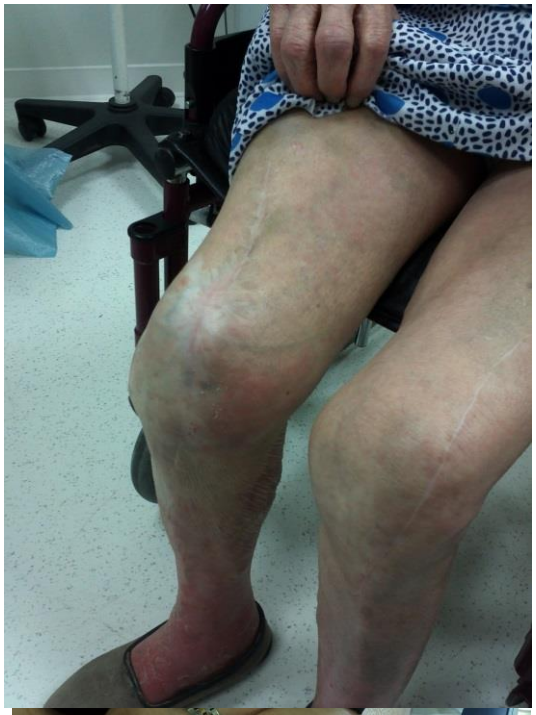

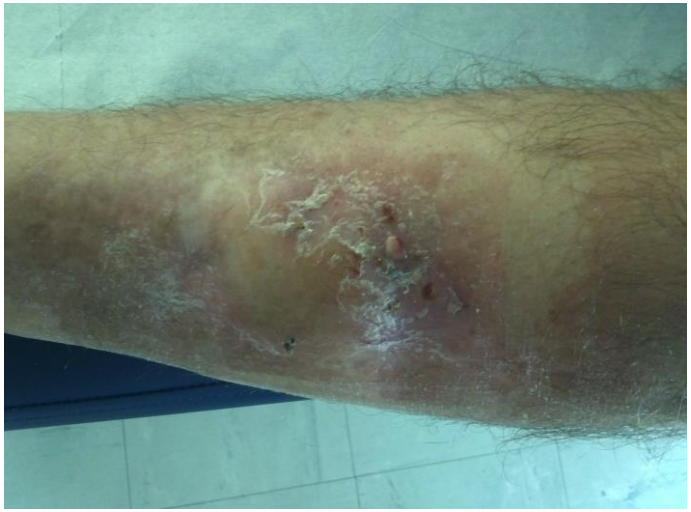

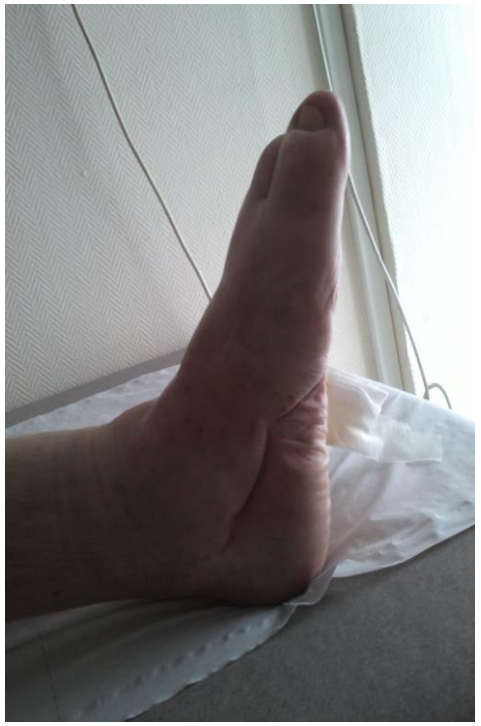

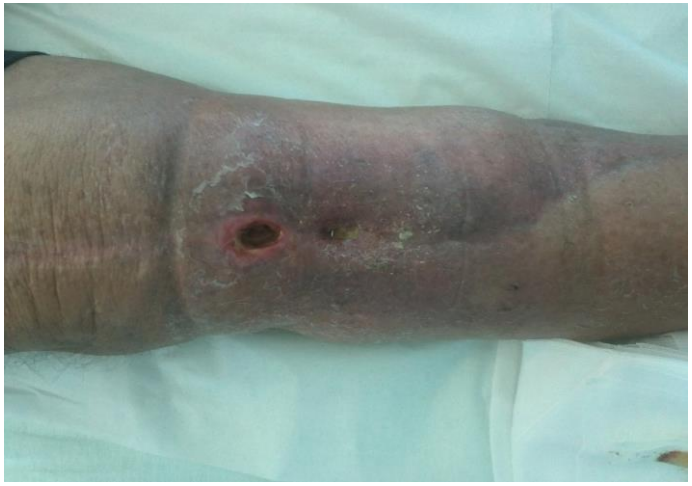

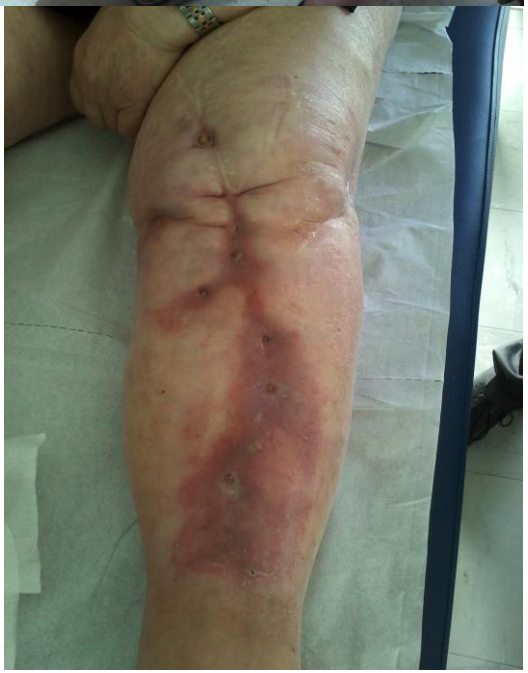

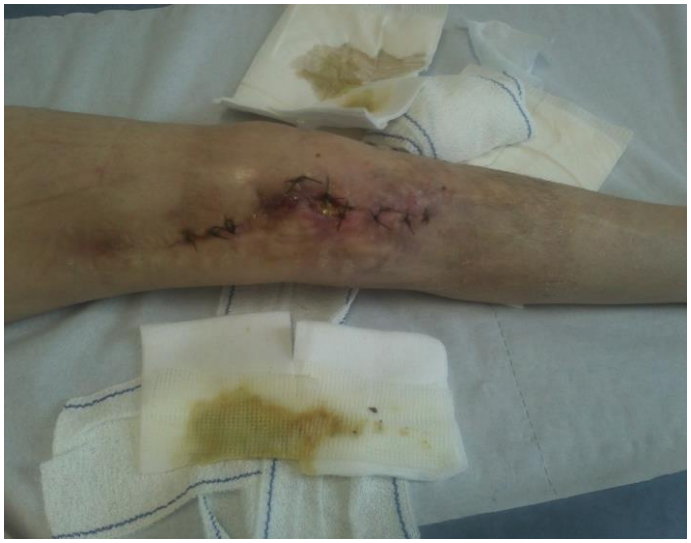

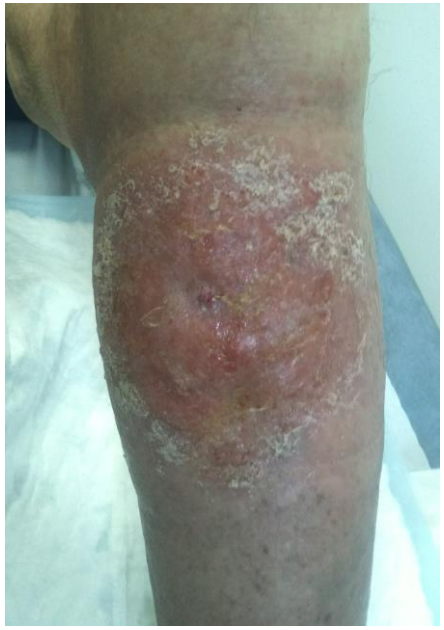

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36th Annual Meeting of the European Bone and Joint Infection Society

7 - 9 September 2017 · Nantes · France

European Bone & Joint Infection Society EBJIS

PHAGE THERAPY FOR BONE AND JOINT INFECTIONS. REPORT OF FRENCH CASES

O.Patey, A.Dublanchet
Department of Infectious and tropical diseases
CHI Lucie et Raymond Aubrac
Villeneuve Saint Georges

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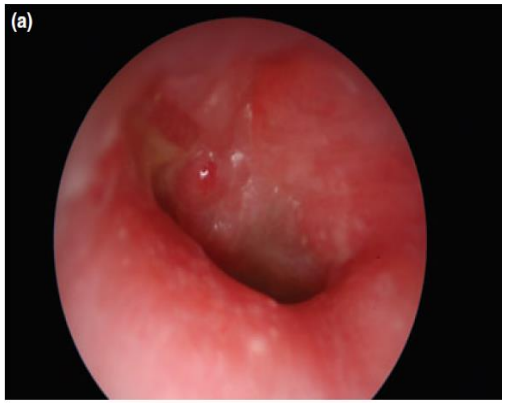
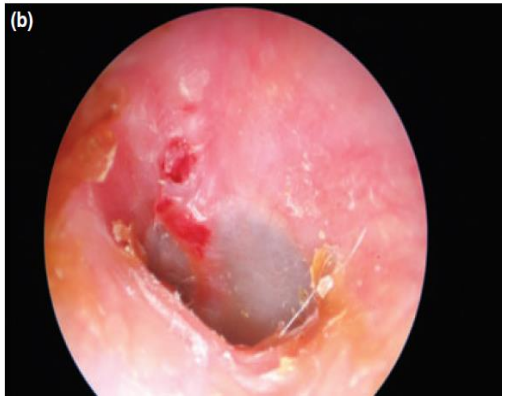
A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy

Wright, A.*, Hawkins, C.H.†, Änggård, E.E.† & Harper, D.R.†





Objectives: To evaluate the efficacy and safety of a therapeutic bacteriophage preparation (Biophage-PA) targeting antibiotic-resistant *Pseudomonas aeruginosa* in chronic *otitis*.

Design: Randomised, double-blind, placebo-controlled Phase I/II clinical trial approved by UK Medicines and Healthcare products Regulatory Agency (MHRA) and the Central Office for Research Ethics Committees (COREC) ethical review process.

Setting: A single specialist university hospital.

Participants: 24 patients with chronic otitis with a duration of several years (2–58). Each patient had, at the time of entry to the trial, an ear infection because of an antibiotic-resistant *P. aeruginosa* strain sensitive to one or more of the six phages present in Biophage-PA. Participants were randomised in two groups of 12 treated with either a single dose of Biophage-PA or placebo and followed up at 7, 21 and 42 days after treatment by the same otologist. Ears were thoroughly cleaned on each occasion and clinical and microbiological indicators measured.

Main outcome measures: Physician assessed erythema/inflammation, ulceration/granulation/polyps, discharge quantity, discharge type and odour using a Visual Analogue Scale (VAS). Patients reported discomfort, itchiness, wetness and smell also using a VAS. Bacterial levels of *P. aeruginosa* and phage counts from swabs were measured initially and at follow-up. At each visit patients were asked about side effects using a structured form. Digital otoscopic images were obtained on days 0 and 42 for illustrative purposes only.

Results: Relative to day 0, pooled patient- and physician-reported clinical indicators improved for the phage treated group relative to the placebo group. Variation from baseline levels was statistically significant for combined data from all clinic days only for the phage treated group. Variation from baseline levels was statistically significant for the majority of the patient assessed clinical indicators only for the phage treated group. *P. aeruginosa* counts were significantly lower only in the phage treated group. No treatment related adverse event was reported.

Conclusion: The first controlled clinical trial of a therapeutic bacteriophage preparation showed efficacy and safety in chronic *otitis* because of chemo-resistant *P. aeruginosa*.

Journal compilation © 2009 Blackwell Publishing Ltd • *Clinical Otolaryngology* 34, 349–357

Corneal Infection Therapy with Topical Bacteriophage Administration

Ali Fadlallah[*,1,3], Elias Chelala[4] and Jean-Marc Legeais[2,3]

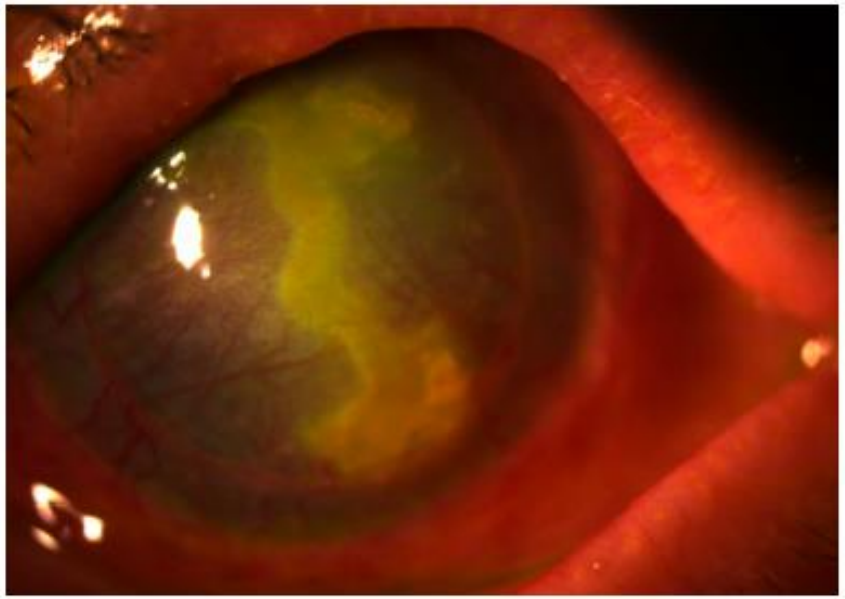


Fig. (1). Slit Lamp photo showing active bacterial keratitis.

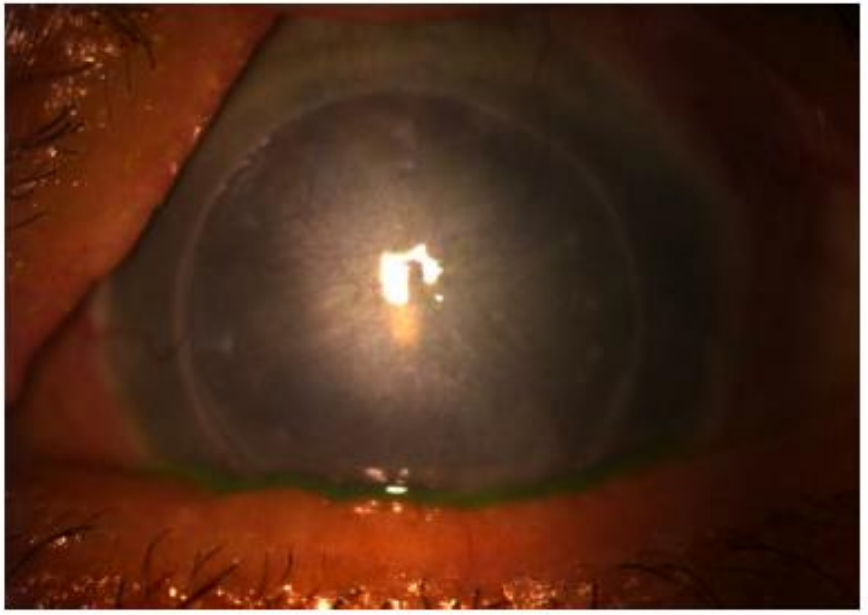


Fig. (2). Slit Lamp photo showing cornea 3 months after topical bacteriophage administration.

Abstract: *Staphylococcus aureus* is a major pathogen in bacterial keratitis, a vision-threatening disease. Although the incidence of S. aureus keratitis varies worldwide, the increasing trend of resistance to certain antibiotics makes this condition an important, global, healthcare concern. We report the case of a 65-year-old woman with nosocomial left-eye corneal abscess and interstitial keratitis.The patient then undergo topical Phage therapy with successful results.

This case indicates that bacteriophage eye-drops may be a novel adjunctive or alternative therapeutic agent for the treatment of infectious keratitis secondary to antibiotic-resistant bacteria. As the incidence of bacterial keratitis is increasing because of inappropriate use of soft contact lenses and infection with multidrug-resistant bacteria, clinical trials are warranted to assess the therapeutic potential of phages in ocular disease, particularly in antibiotic-resistant cases.

The Open Ophthalmology Journal, 2015, *9*, 167-168

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Use of bacteriophages in the treatment of colistin-only-sensitive *Pseudomonas aeruginosa* septicaemia in a patient with acute kidney injury—a case report

Serge Jennes[1], Maia Merabishvili[2], Patrick Soentjens[3], Kim Win Pang[3], Thomas Rose[1], Elkana Keersebilck
Olivier Soete[1], Pierre-Michel François[1], Simona Teodorescu[1], Gunther Verween[2], Gilbert Verbeken[2],
Daniel De Vos[2] and Jean-Paul Pirnay[2*]

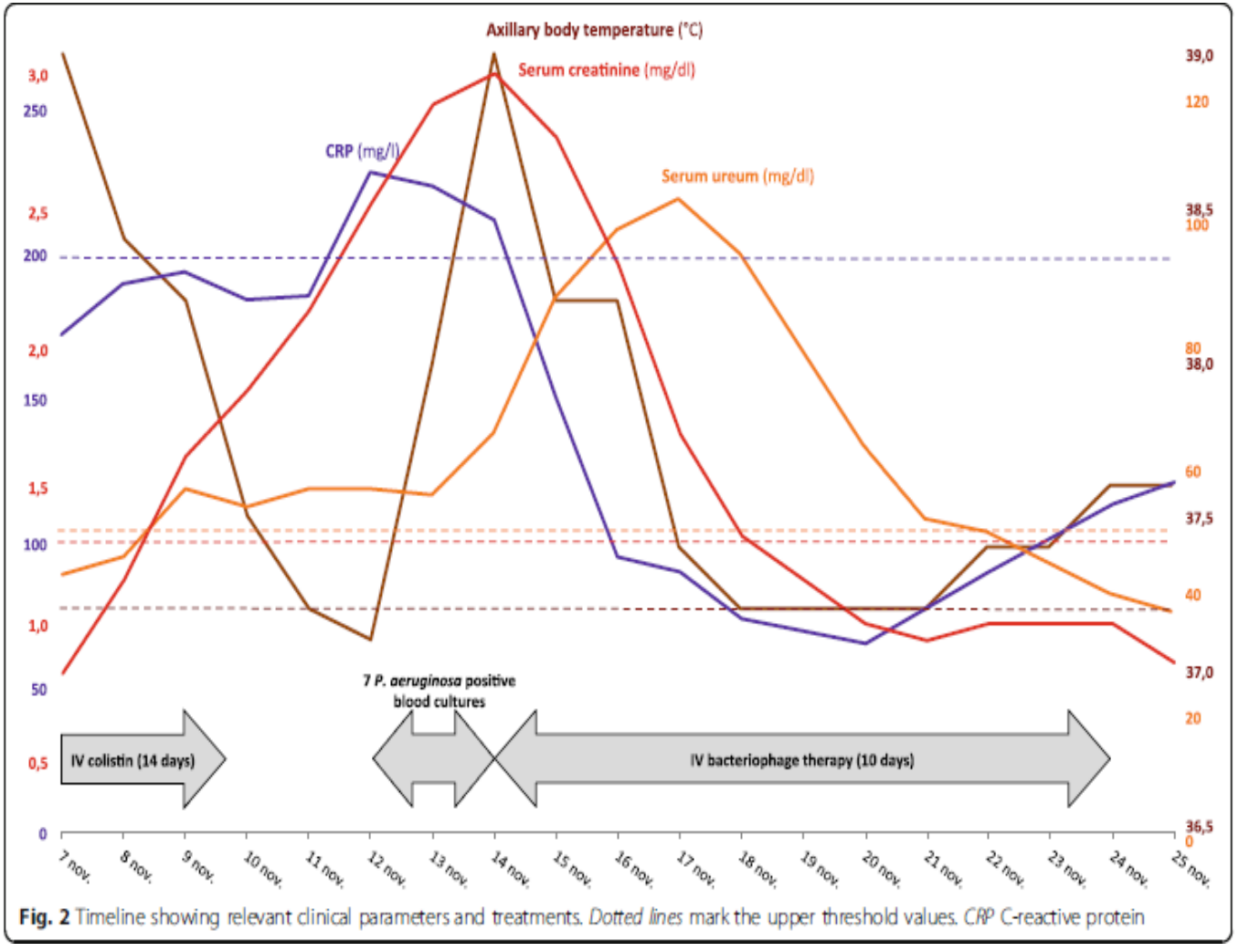

Fig. 2 Timeline showing relevant clinical parameters and treatments. *Dotted lines* mark the upper threshold values. *CRP* C-reactive protein

Fig. 1 Large pressure sores in the sacral and spinal back areas. Situation on 17 November 2016

Jennes *et al. Critical Care* (2017) 21:129
DOI 10.1186/s13054-017-1709-y

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Phage Therapy in a 16-Year-Old Boy with Netherton Syndrome

Pikria Zhvania[1], Naomi Sulinger Hoyle[1], Lia Nadareishvili[1], Dea Nizharadze[1] and Mzia Kutateladze[2]*

[1] *Eliava Phage Therapy Center, Tbilisi, Georgia,* [2] *G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia*

FIGURE 1 | Local application of phage using gauze and liquid phage preparations.

TABLE 2 | Bacteriophage preparations (produced by Eliava Biopreparations Ltd.) used for treatment.

Phage preparation	Composition	Form	Titer	Local application	Oral	Eye	Nose
Pyo bacteriophage	Phage lysates of *Streptococcus* spp., *Staphylococcus* spp., *E. Coli*, *Pseudomonas aeruginosa*, and *Proteus* spp	Liquid	$\approx 10^7$	+	+	+	+
Staphylococcus bacteriophage	Phage lysates of *Staphylococcus* spp.	Liquid, cream	$\approx 10^7$	+	+	–	–
Fersis bacteriophage	Phage lysates of *Staphylococcus* spp., *Streptococcus* spp.	Liquid	$\approx 10^7$	+	+	+	+

INFORMED CONSENT

Bacteriophage preparations are a registered drug in Georgia. Treatment with phage is not considered to be experimental in Georgia. Despite this, our patients are informed that bacteriophage preparations are not registered drugs in Europe and the United States. The parents of our patient agree to have their son's information and photographs published in scientific articles.

ETHICS STATEMENT

Phage therapy is not an experimental therapy in Georgia. The phage preparations used are registered drugs in Georgia, and the treating physicians are licensed in Georgia.

frontiers in Medicine

CASE REPORT
published: 03 July 2017
doi: 10.3389/fmed.2017.00094

ansm
Agence nationale de sécurité du médicament et des produits de santé

DÉCISION DG n° 2016-11

du 13 JAN. 2016 **portant création d'un Comité scientifique spécialisé temporaire « Phagothérapie » à l'Agence nationale de sécurité du médicament et des produits de santé**

Article 2 : Le comité scientifique spécialisé temporaire « Phagothérapie » est chargé de donner un avis quant aux situations cliniques pouvant justifier d'un accès précoce aux bactériophages et aux prérequis nécessaires pour une mise à disposition précoce dans le cadre d'autorisations temporaires d'utilisation (ATU) ou d'essais cliniques.

Agence nationale de sécurité du médicament et des produits de santé

COMITE SCIENTIFIQUE SPECIALISE TEMPORAIRE

CSST201611011- Phagothérapie

Jeudi 24 mars 2016 de 9h30 à 17h00, en salle A011

DÉCISION DG n° 2016-11

du 13 JAN. 2016 portant création d'un Comité scientifique spécialisé temporaire « Phagothérapie » à l'Agence nationale de sécurité du médicament et des produits de santé

Programme de séance		
	Sujets abordés	**Action** (pour audition, information, adoption ou discussion)
1.	**Introduction**	
1.1	Adoption de l'ordre du jour	Pour adoption
2.	**Dossier thématique**	
2.1	PHAGOTHERAPIE Elaboration d'une position quant aux situations cliniques pouvant justifier d'un accès précoce aux bactériophages, et détermination de prérequis nécessaires pour une mise à disposition précoce des bactériophages	Pour discussion
5.	**Tour de Table**	Pour discussion

Question posée N°1	Quelles sont les situations de besoin ?
Question posée N°2	Quels sont les domaines et les objectifs thérapeutiques à cibler ?
Question posée N°3	Quels sont les pré-requis nécessaires pour une mise à disposition précoce des bactériophages ?

CENTRE HOSPITALIER INTERCOMMUNAL
VILLENEUVE-SAINT-GEORGES
Lucie & Raymond AUBRAC

GEEPhage Phagothérapie

QUE FAIRE?

Création rapide de structures de « référence » au niveau de l'Union Européenne avec missions bien définies comme nous le réclamons depuis plusieurs années avec PHAGE.org

P-H-A-G-E.org

Phages for Human Applications Group Europe vzw
Militair Hospitaal Koningin Astrid
C DIS/Site NOH, Blok C, 1ste verdieping
Lokaal 1.391
Bruynstraat 1
1120 BRUSSEL

Existence de:

- CNR par germes
- CNR par pathologies: Infections ostéo-articulaires
- CNR Maladies rares

Situation particulière de la phagothérapie car:

- il s'agit d'une thérapeutique
- s'appliquant à de nombreuses pathologies d'organe
- destinée à traiter de nombreuses infections souvent nosocomiales
- devant tenir compte de l'expérience de l'antibiothéra

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

MINISTÈRE
DES AFFAIRES SOCIALES
ET DE LA SANTÉ

Centres de référence labellisation, structures spécialisées

DGOS PF2 – v1 - Mai 2012

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MISSIONS D'UN CNR

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1. **Mission de prise en charge de recours** de niveau au moins régional ou interrégional, cette prise en charge étant pluridisciplinaire et globale ;

2 **Mission de coordination** : le centre est une tête de pont. Il constitue des filières (d'amont et d'aval) sur son territoire, dresse un annuaire de ses correspondants, assure le lien avec les usagers et l'administration, réalise des actions d'information et de communication ;

3 **Mission d'expertise** : en lien avec la prise en charge de recours, le centre organise des réunions de concertation pluridisciplinaires (RCP). Il assure la réalisation et la diffusion de procédures et protocoles, réalise un recueil épidémiologique. Il s'assure de la qualité des prises en charge et forme les professionnels ;

4 **Mission d'enseignement** : le centre anime, promeut et participe à la réalisation d'enseignements universitaires et post-universitaires ;

5 **Mission de recherche** : le centre anime, promeut et participe à la réalisation de programmes de recherche clinique, translationnelle et fondamentale, sur les soins, en épidémiologie, en matière de qualité ;

De plus, un centre de référence doit être **bien identifié** et **visible**.

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PHAGE THERAPY CENTER

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Phagothérapie

ELIAVA INSTITUTE
TBILISSI
GEORGIA

DSMZ

HIRZFELD INSTITUTE
WROCLAW
POLAND

EUROPEAN PHAGES BANK

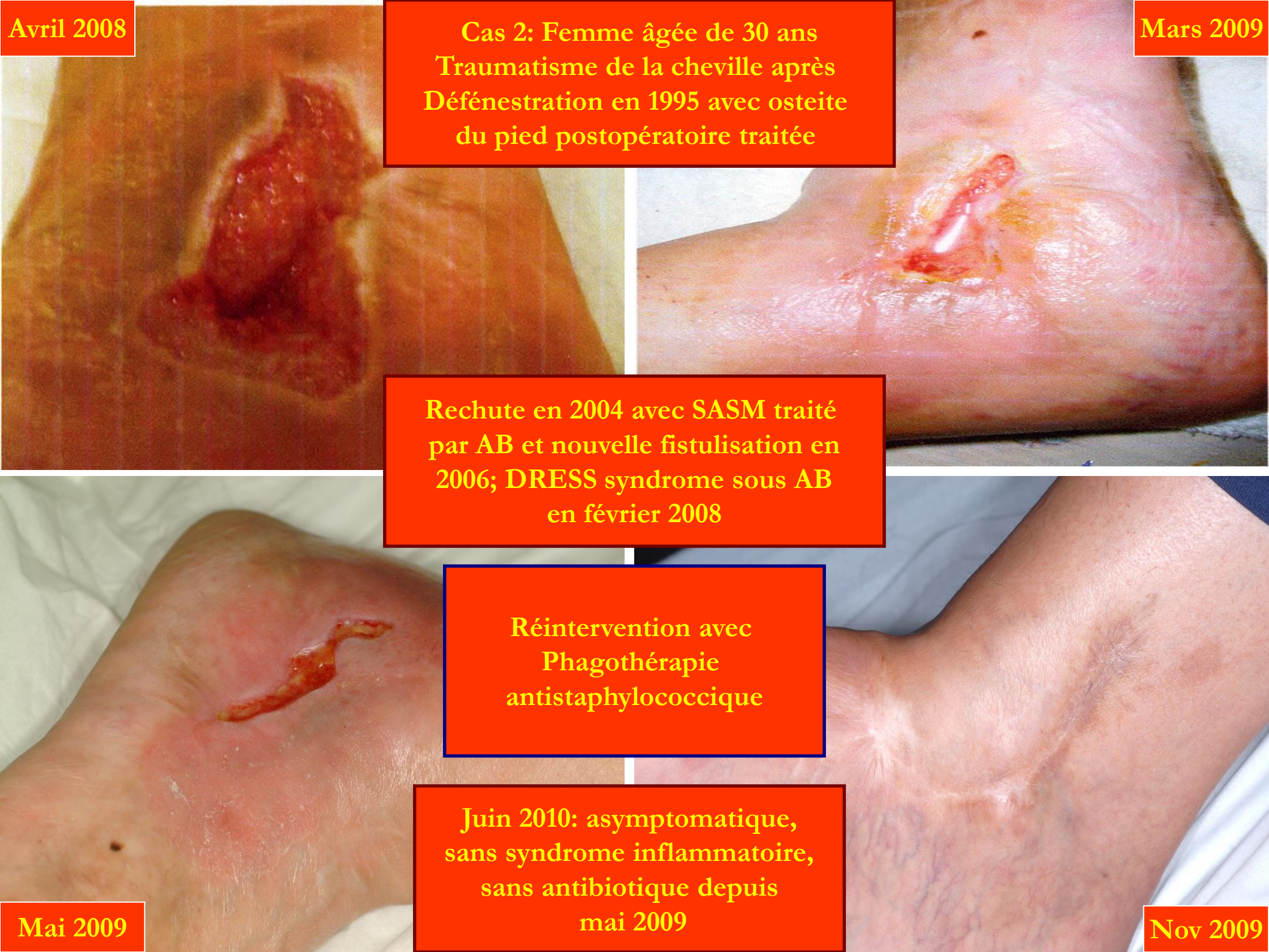
DSMZ (Deutsche Sammlung von Mikroorganismen and Zellkulturen)
GERMANY

CHI VILLENEUVE
SAINT GEORGES
FRANCE

USA
Eliava Phages NY

M BALS INSTITUTE
BUCAREST
ROMANIA ?

QUEEN ASTRID
MILITARY HOSPITAL
BELGIAN


Avril 2008
Cas 2: Femme âgée de 30 ans
Traumatisme de la cheville après
Défénestration en 1995 avec osteite
du pied postopératoire traitée
Mars 2009
Rechute en 2004 avec SASM traité
par AB et nouvelle fistulisation en
2006; DRESS syndrome sous AB
en février 2008
Réintervention avec
Phagothérapie
antistaphylococcique
Juin 2010: asymptomatique,
sans syndrome inflammatoire,
sans antibiotique depuis
mai 2009
Mai 2009
Nov 2009

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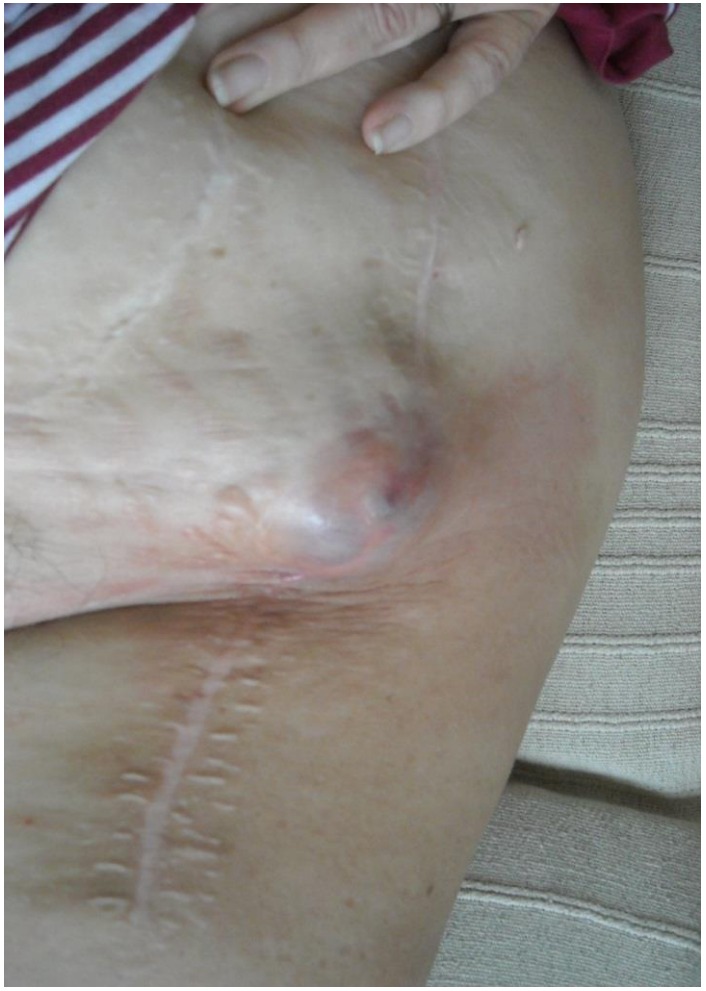
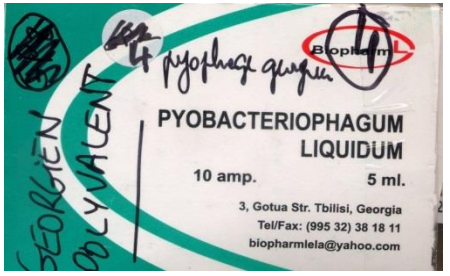
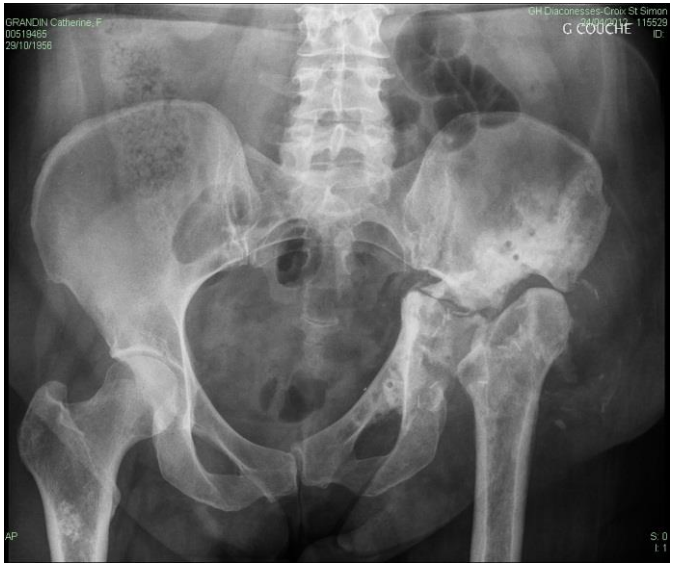
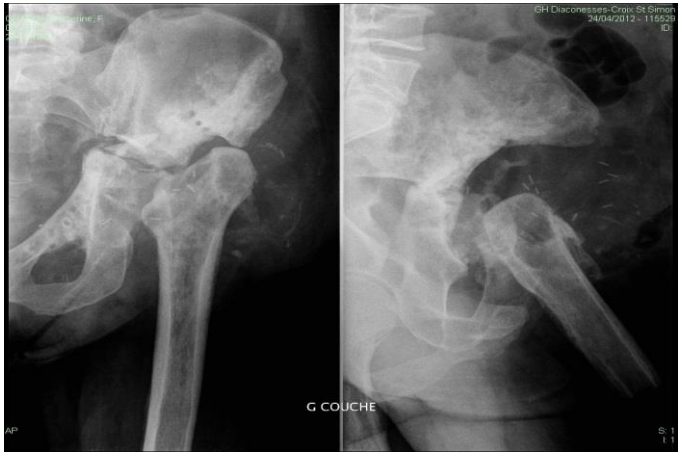
Prélèvement	Référence	Date prélèvt	late demand	Souche testée	Date-test	Demandeur	1 ASR1	2 ASR2	3 RP3	4 GP4	5 GP5	6 ASG6	8 RP8
Plaie chirurgicale	1209 21 1566		19/09/2012	*Staphylococccus aureus*	12/10/2012	Dr J. Philippe Damman Médecin généraliste Médecin trait. 65230 TRIE/BAISE LABM 31210 MONTREJEAU	S	R	R			R	
	1205160279	16/05/2012	25/09/2012	*Pseudomonas aeruginosa*	12/10/2012	Dr MAYAN Rémi CH Angoulème 16000			R	R	S		S
	1205160279	16/05/2012	25/09/2012	*Enterococcus avium*	12/10/2012	Dr MAYAN Rémi CH Angoulème 16000			R	R	S faible		S
	1209200335	16/05/2012	25/09/2012	*Staphylococcus aureus*	12/10/2012	Dr MAYAN Rémi CH Angoulème 16000	S	R	R			S	
	1209200335	16/05/2012	25/09/2012	*Proteus mirabilis*	12/10/2012	Dr MAYAN Rémi CH Angoulème 16000			R	R	S faible		S faible









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NECESSITE DE CREATION D'UNE BANQUE DE BACTERIOPHAGES

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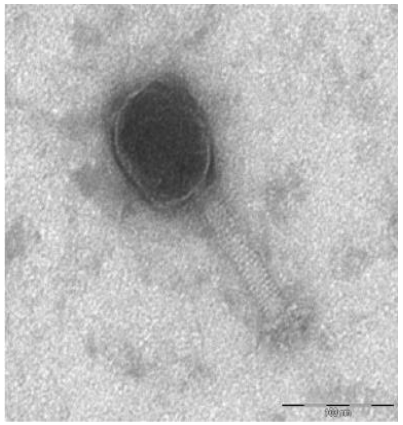
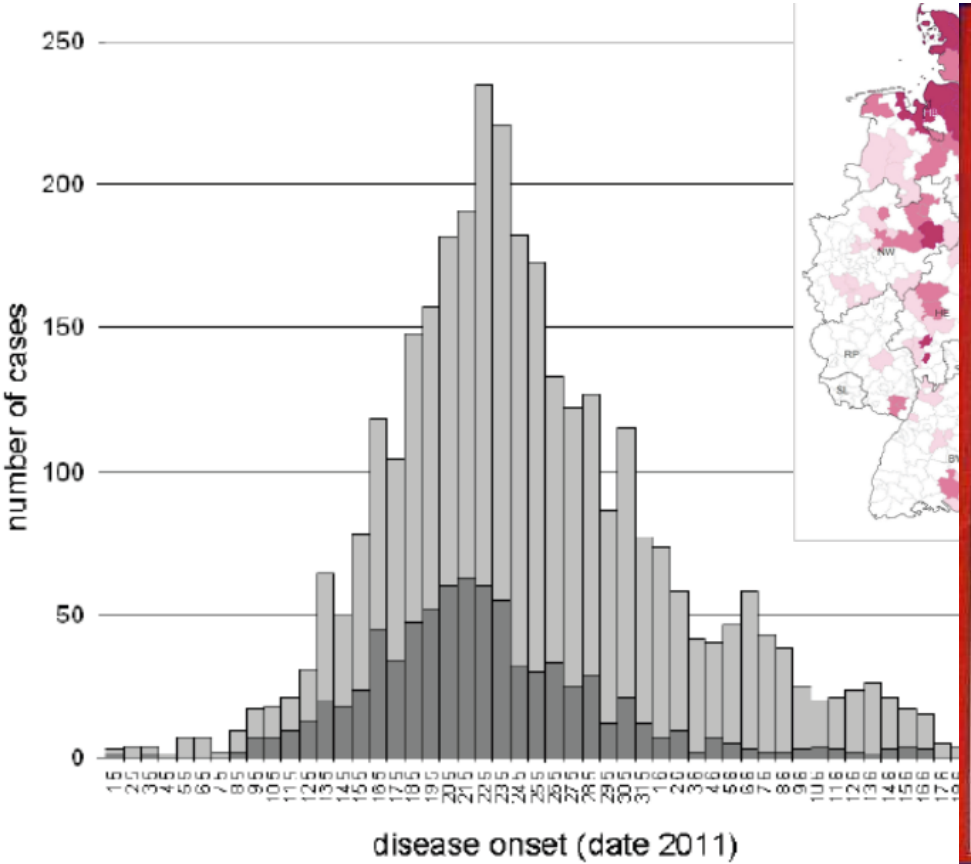
Selection and Characterization of a Candidate Therapeutic Bacteriophage That Lyses the *Escherichia coli* O104:H4 Strain from the 2011 Outbreak in Germany



Maia Merabishvili[1,2,3], Daniel De Vos[1], Gilbert Verbeken[1], Andrew M. Kropinski[4,5], Dieter Vandenheuvel[6], Rob Lavigne[6], Pierre Wattiau[7], Jan Mast[8], Catherine Ragimbeau[9], Joel Mossong[9], Jacques Scheres[10,11], Nina Chanishvili[2], Mario Vaneechoutte[3], Jean-Paul Pirnay[1]*

1 Laboratory for Molecular and Cellular Technology, Queen Astrid Military Hospital, Brussels, Belgium, **2** Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi, Georgia, **3** Laboratory of Bacteriology Research, Ghent University, Ghent, Belgium, **4** Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Ontario, Canada, **5** Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada, **6** Laboratory of Gene Technology, KU Leuven, Heverlee, Belgium, **7** Unit of Highly Pathogenic & Foodborne Zoonoses, Veterinary and Agrochemical Research Centre, Brussels, Belgium, **8** Electron Microscopy Unit, Veterinary and Agrochemical 8 Research Centre, Brussels, Belgium, **9** Surveillance and Epidemiology of Infectious Diseases, Laboratoire National de Santé, Luxembourg, Luxembourg, **10** Maastricht University Medical Centre, Maastricht, The Netherlands, **11** European Centre for Disease Prevention and Control, Stockholm, Sweden



Natural bacteriophages against multidrug resistant (MDR) bacteria

Patey O[1]., Breuil J.[2], Alavidze Z[3]., Mingot N[2]., Dublanchet A. [1,2]

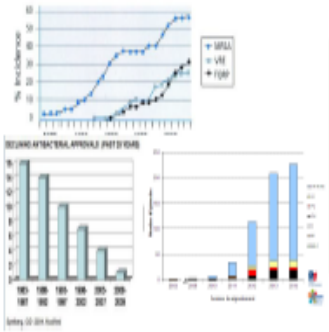
1. Department of infectious and tropical diseases 2. Laboratory of microbiology, Villeneuve Saint Georges Hospital, France 3. Georges Eliava Institute, Tbilisi Georgia

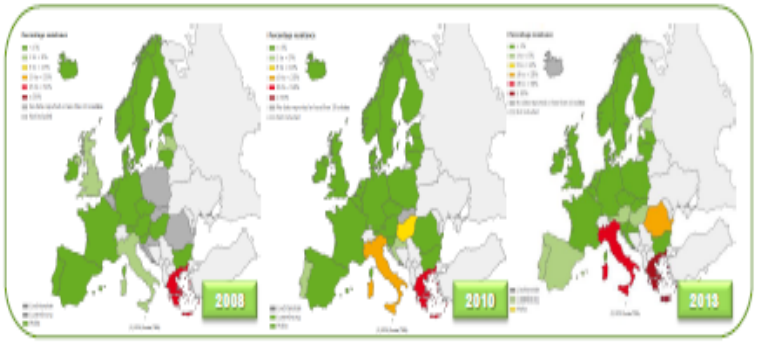
Introduction

Phages were discovered in 1915 by F. W. Twort and F. d'Herelles who isolated and used them for the first time in 1919 for the treatment of dysenteria. Since that time, many bacterial infection have been treated with phages around the world. They were replaced by antibiotics in Western Europe, but continued to be used in East Europa.

During the last years we observed a dramatic increase of MDR bacteria, especially carbapenemase producing enterobacteria , associated with nosocomial outbreaks.

Such an outbreak due to a multidrug resistant strain of oxa 48 producing *Klebsiella pneumoniae (K. pneumoniae)* occurred at Villeneuve Saint Georges Hospital in 2010-2011 with 3 waves (table I)





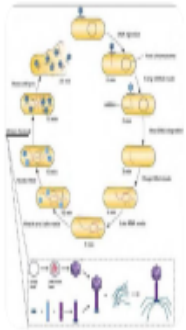


Table1 :Outbreak in Villeneuve Saint Georges Hospital

WAVES	1	2	3
CASES	10	2	2
DEATH	7	0	0
CONTACTS	283	56	37

14 patients with this strain were identified (with or without infection).and 283 contacts. The *K. pneumoniae* oxa 48 was sent to the Georges Eliava Institute, Tbilissi, Géorgia, for the isolation of a specific lytic phage.

Results

Antibiotic susceptibility of the epidemic strain of our hospital:

K. pneumoniae strain was multidrug resistant, being only susceptible to colimycine and of intermediate susceptibility to Amikacine and tygecycline.

Phage efficacy

All our isolates were susceptible to the specific phage.

This efficacy was found for 3 of the 4 French clonal strains. Commercialy available phages cocktails

Comments

These results show a great efficacy of the specific anti *K pneumoniae* phage isolates of Eliava Institute against our epidemic strain. Other French epidemic strains of *K. pneumoniae* oxa 48 were also sensitive but such an efficacy was not found with 3 commercially available

Matérial ar

Phage isolation

One ml bacteria
and 10ml concer
hours . Centrifu
recovered using
To test the sens
Bacterial strain
LB or TSB agar

Phage products

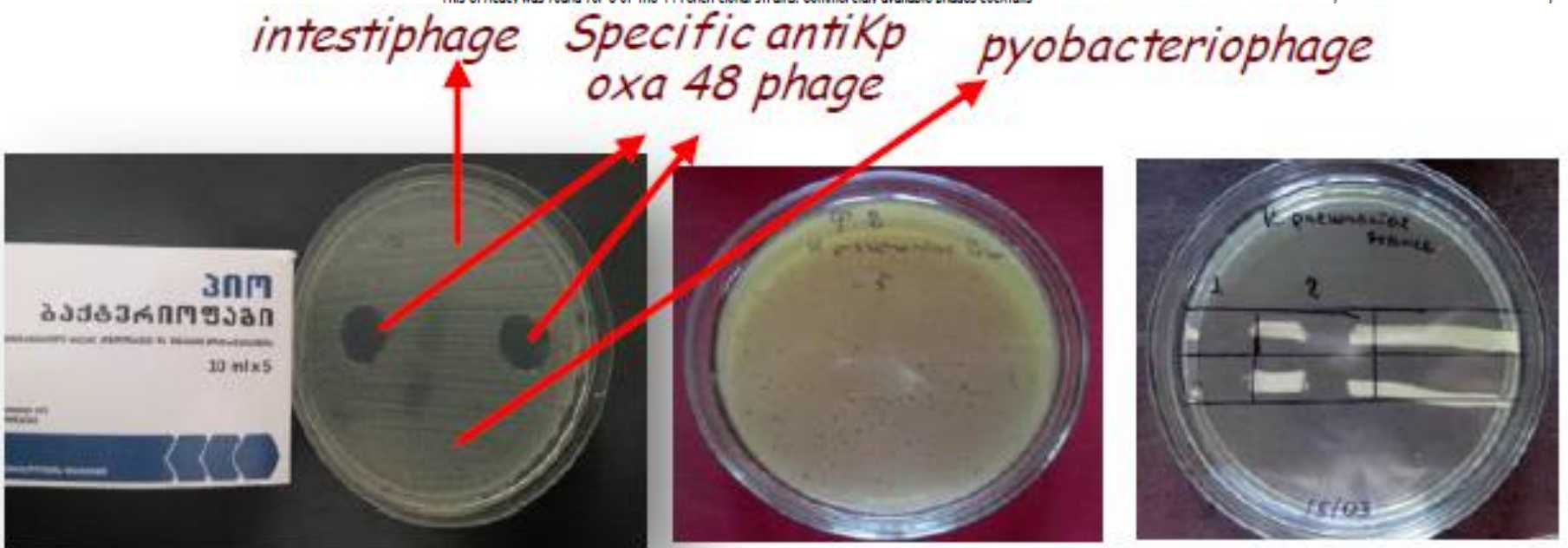
Three commerc
2 polyvalent ph
and one contain

Strains

All the 14 *K.pne*
clonal *K.pneum*
Center, Kremli
outbreak in Bas

Phage effica

To test the pha
Muller Hinton agar on which a *K.pneumoniae* dilution of 0,2 Mc Farland had been spread by swabbing.
The test was interpreted after 24 hours.



National and European Authority and international health organisation (WHO) should be more implicated to develop research and trial with phages.

strains of K. pneumoniae send to our microbiological Laboratory.

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Phagothérapie

Des hôpitaux débordés par tuberculeux d'Europe de l'E

Par Yves Mamou - le 23/01/2013

INFO LE FIGARO - Depuis quelques mois, des dizaines de Géorgiens, Tchétchènes et Russes, atteints d'une tuberculose ultrarésistante, débarquent en France. Outre le coût élevé de leur prise en charge, le risque de contagion inquiète les autorités sanitaires.

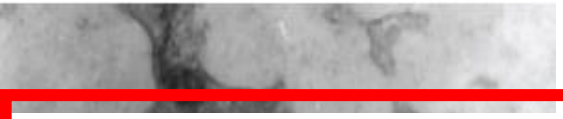
Une réponse: les PHAGES

TB: the return of the phage. A review of fifty years of mycobacteriophage research

INT J TUBERC LUNG DIS 3(3):179–184

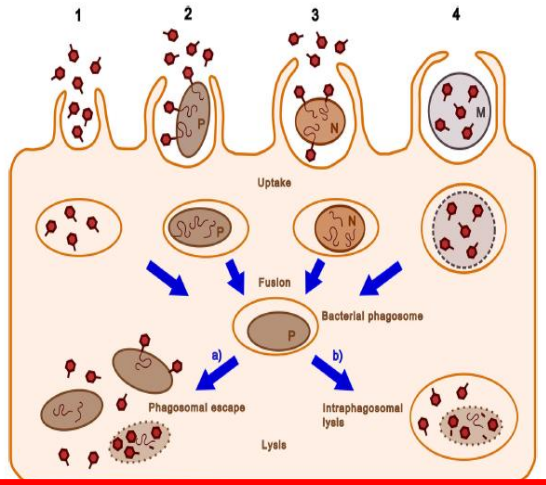
R. McNerney

Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK



A Question of Attire: Dressing Up Bacteriophage Therapy for the Battle Against Antibiotic-Resistant Intracellular Bacteria

Anita Nieth · Cyprien Verseux · Winfried Römer



Effect of mycobacteriophage to intracellular mycobateria *in vitro*

PENG Li, CHEN Bao-wen, LUO Yong-ai and WANG Guo-zhi

A12. ISOLATION AND CHARACTERIZATION OF SIX NOVEL MYCOBACTERIOPHAGES AND INVESTIGATION OF THEIR ANTIMICROBIAL POTENTIAL IN MILK

Lorraine Endersen[1], Aidan Coffey[1]*, Horst Neve[2], Olivia McAuliffe[3], R. Paul Ross[3] and Jim O'Mahony[1]

CENTRE HOSPITALIER INTERCOMMUNAL
VILLENEUVE-SAINT-GEORGES
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Pharm Res. 2017 Jun 23. doi: 10.1007/s11095-017-2213-4. [Epub ahead of print]

Anti-Tuberculosis Bacteriophage D29 Delivery with a Vibrating Mesh Nebulizer, Jet Nebulizer, and Soft Mist Inhaler.

Carrigy NB[1], Chang RY[2], Leung SSY[2], Harrison M[3], Petrova Z[4], Pope WH[4], Hatfull GF[4], Britton WJ[5], Chan HK[2], Sauvageau D[3], Finlay WH[1], Vehring R[6,7].

Author information

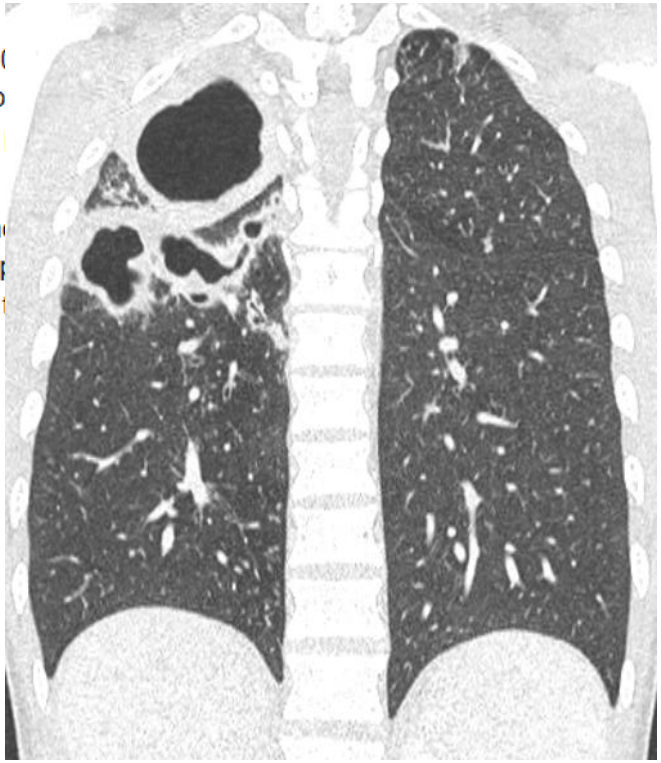
Abstract

PURPOSE: To compare titer reduction and delivery rate of active anti-tuberculosis bacteriophage (phage) D29 with three inhalation devices.

METHODS: Phage D29 lysate was amplified to a titer of 11.8 ± 0.3 $\log_{10}$(pfu/mL) and diluted 1:100 in isotonic saline. Filters captured the aerosolized saline D29 preparation emitted from three types of inhalation devices: 1) vibrating mesh nebulizer; 2) jet nebulizer; 3) soft mist inhaler. Full-plate plaque assays, performed in triplicate at multiple dilution levels with the surrogate host Mycobacterium smegmatis, were used to quantify phage titer.

RESULTS: Respective titer reductions for the vibrating mesh nebulizer, jet nebulizer, and soft mist inhaler were (
0.6 ± 0.3 $\log_{10}$(pfu/mL). Active phage delivery rate was significantly greater ($p < 0.01$) for the vibrating mesh neb
pfu/min) than for the jet nebulizer ($5.4 \times 10^4 \pm 1.3 \times 10^4$ pfu/min). The soft mist inhaler delivered $4.6 \times 10^6 \pm 2.0 \times 10^6$
actuator dose.

CONCLUSIONS: Delivering active phage requires a prudent choice of inhalation device. The jet nebulizer was n
aerosolizing phage D29 under the tested conditions, due to substantial titer reduction likely occurring during dro
mesh nebulizer is recommended for animal inhalation studies requiring large amounts of D29 aerosol, whereas
useful for self-administration of D29 aerosol.



KEYWORDS: Mycobacterium tuberculosis; aerosol; nebulization; phage therapy; titer reduction

PMID: 28646325 DOI: 10.1007/s11095-017-2213-4

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Burns. 2017 May 11. pii: S0305-4179(17)30206-1. doi: 10.1016/j.burns.2017.03.029. [Epub ahead of print]

Liposome loaded phage cocktail: Enhanced therapeutic potential in resolving Klebsiella pneumoniae mediated burn wound infections.

Chadha P[1], Katare OP[2], Chhibber S[3].

Author information

Abstract

BACKGROUND: Klebsiella pneumoniae is one of the predominant pathogens in burn wound infections, and prevalence of multidrug resistant strains has further complicated the situation. An increased interest in phage therapy as a means of combating infection has been accruing in recent years. In order to overcome the drawbacks associated with phage therapy, the present study was conducted to evaluate the potential of liposomes as a delivery vehicle for phage in the treatment of burn wound infection.

METHODS: Burn wound infection with Klebsiella pneumoniae B5055 was established in BALB/c mice. The therapeutic efficacy of free phage cocktail in comparison to liposome entrapped phage cocktail in resolving the course of burn wound infection in mice was evaluated.

RESULTS: The results depicted that mice treated with liposomal entrapped phage cocktail showed higher reduction in bacterial load in blood and major organs. This was accompanied with faster resolution of the entire infection process as compared to non-liposomal free phage cocktail. The liposomes increased phage retention time in vivo thus potentiating efficacy. Liposomal phage preparation was able to protect all the test animals from death even when there was a delay of 24h in instituting the therapy.

CONCLUSION: The results showed the potential of liposome entrapped phage cocktail for treating Klebsiella pneumoniae mediated infections. Thus, this strategy can serve as an effective approach for treating Klebsiella mediated burn wound infections in individuals who do not respond to conventional antibiotic therapy.

KEYWORDS: Bacteriophage; Burns; Infection; Klebsiella pneumoniae; Liposomes

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MISSIONS D'UN CNR

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PHOSA

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

5 **Mission de recherche** : le centre anime, promeut et participe à la réalisation de programmes de recherche clinique, translationnelle et fondamentale, sur les soins, en épidémiologie, en matière de qualité ;

De plus, un centre de référence doit être **bien identifié** et **visible**.

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EQUIPES MULTIDISCIPLINAIRES

CLINICIENS, MICROBIOLOGISTES, ECOLOGISTES PHARMACIENS, CHERCHEURS, JURISTES VETERINAIRES, SOCIETE CIVILE, ETHIQUE

COOPERATION EUROPEENNE : RESEAU DE CNR

P-H-A-G-E.org

Phages for Human Applications Group Europe vzw
Militair Hospitaal Koningin Astrid
C DIS/Site NOH, Blok C, 1ste verdieping
Lokaal 1.391
Bruynstraat 1
1120 BRUSSEL

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NÉCESSITE D'UNE SURVEILLANCE ÉCOLOGIQUE

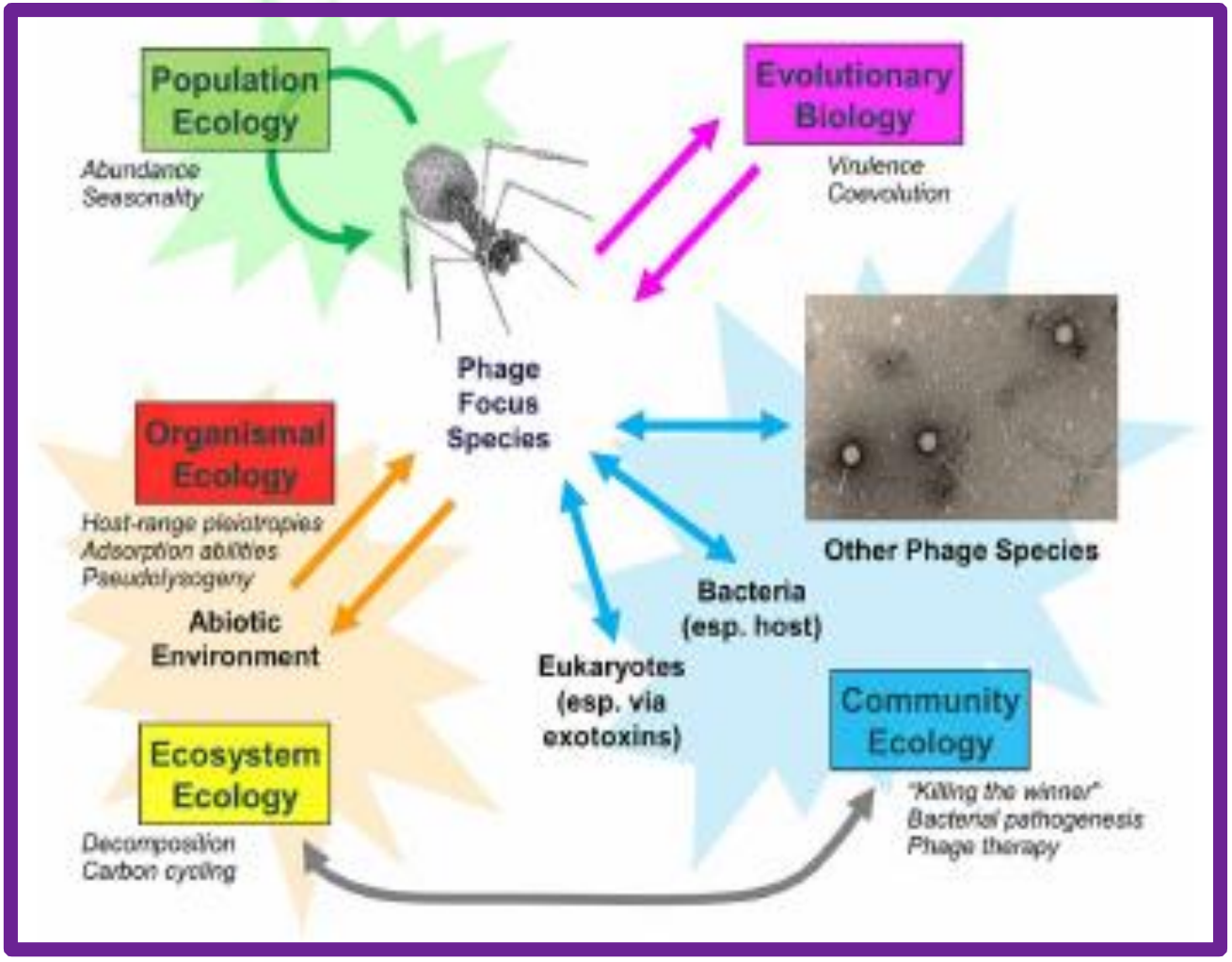


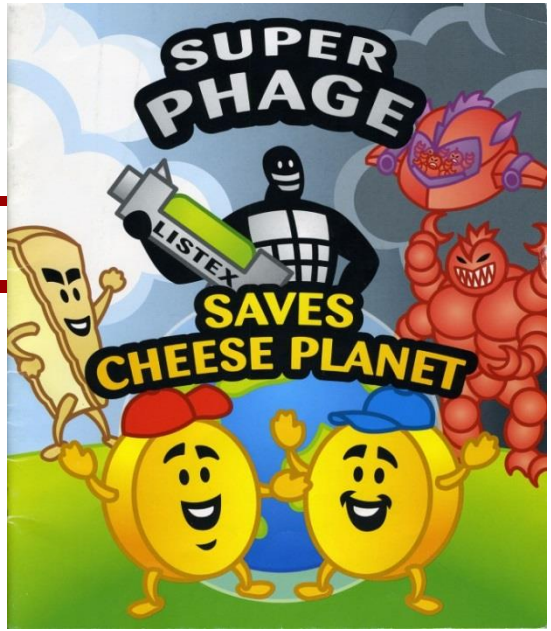
FIGURE 1 | Bacteriophage population, community, and ecosystem impacts. Examples of phage-associated changes are italicized. Adapted from Abedon (2009).

FDA approved products

(GRAS status i.e. Generally Recognized as Safe)

- 2006 August - **ListShieldTM** ready-to-eat meat and poultry products, Intralytix, USA
- 2006 October - **Listex P100**, EBI Food Safety, Netherlands
- 2011- **EcoShield** against *E.coli O157*

2006 - AgriPhage, Omnilytics, EPA registration



2014 : SALMONEX

BACTERIOPHAGE ET AGROALIMENTAIRE

CENTRE HOSPITALIER INTERCOMMUNAL
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ansm
Agence nationale de sécurité du médicament et des produits de santé

Mars 2014

La transplantation de microbiote fécal et son encadrement dans les essais cliniques

TMF= coprophagie médicalement assistée .

•Dilutions (non standardisée): 50 à 60 g de selles/ 200 à 300ml de solutions (eau, sérum salé, lait,...). Moyens Techniques variables.

10 9 bactéries par gramme de selles
10 à 100 fois plus de bac-tériophages

1. Généralités et Cadre réglementaire applicable

En France

- Selon le Code de la santé publique
  - ➢ Pas de statut particulier pour le microbiote fécal
  - ➢ Art.L. 5111-1 = définition d'un médicament
  - ➢ OR microbiote fécal utilisé à visée curative DONC considéré comme un MEDICAMENT
- Stade précoce de développement + Pas d'AMM DONC cadre législatif applicable aux
  - ➢ Préparations magistrales et hospitalières
  - ➢ Mdcts expérimentaux destinés à 1 essai clinique
  - ➢ Art. L. 5121-1 et L. 5121-1-1 du Code de la SP

Au niveau international

- Hétérogénéité sur le statut du microbiote fécal
- USA : médicament
- UE : Tissu (Royaume-Uni, Danemark et Pays-Bas)

(ANSM 2014)

CENTRE HOSPITALIER INTERCOMMUNAL
VILLENEUVE-SAINT-GEORGES
Lucie & Raymond AUBRAC

MERCI POUR VOTRE ATTENTION

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