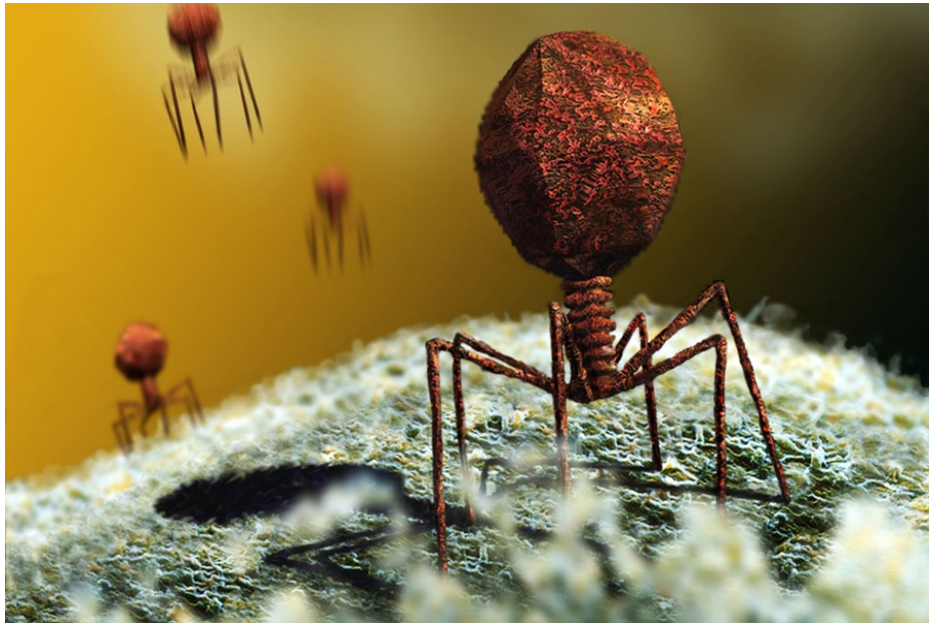
BACTERIOPHAGES
Clinical Applications



X. Wittebole, MD
Critical Care Department

Cliniques universitaires
SAINT-LUC
UCL BRUXELLES

BACTERIOPHAGES

- The most abundant and ubiquitous organism on Earth
- 10^4 – 10^8 / ml particles in aquatic systems
- 10^9 / g particles in soil
- 10^{32} particles on Earth
- > 6300 different bacteriophages discovered and described

  6196 bacterial viruses, 88 aracheal viruses

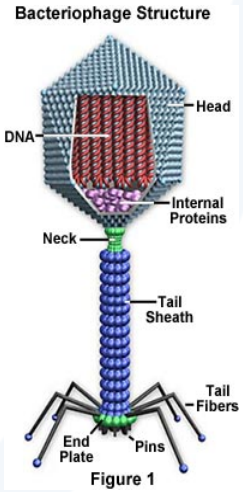


Figure 1

Small viruses able at killing bacteria while they do not affect other cell lines

1917 : FELIX D'HERELLE

- Paris vs Quebec ? (1875 – 1949)
- Lived in Paris, Lille, Leiden, Montreal
- Never graduated in Medicine
- Worked as a microbiologist in Guatemala
- Travelled around the world
- Found that locust were killed by Coccobacillus acridorium
- Found « plaques » in culture of dysentery bacilli

***On an invisible microbe antagonistic to dysentery bacilli*. Note by M. F. d'Herelle, presented by M. Roux. Comptes Rendus Academie des Sciences 1917; 165:373–5**

MICROBIOLOGIE. — *Sur un microbe invisible antagoniste des bacilles dysentériques*. Note (3) 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 ce jour, avec la première souche isolée, plus de 50 réensemencements successifs. L'expérience suivante donne d'ailleurs la preuve visible que l'action antagoniste est produite par un germe vivant : si l'on ajoute à une culture de Shiga une dilution d'une culture précédente lysée, de façon que la culture de Shiga n'en contienne qu'un millionième environ, et si, immédiatement après, on étale sur gélose inclinée une gouttelette de cette culture on obtient, après incubation, une couche de bacilles dysentériques présentant un certain nombre de cercles d'environ 1^{mm} de diamètre, où la culture est nulle; ces points ne peuvent représenter que des colonies du microbe antagoniste : une substance chimique ne pourrait se concentrer sur des points définis. En opérant sur des quantités mesurées, j'ai pu voir qu'une

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

MICROBIOLOGIE. — *Sur un microbe invisible antagoniste des bacilles dysentériques*. Note (³) de M. **F. d'Herelle**, présentée par M. Roux.

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

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

D'Herelle F. Bull N Y Acad Med.1931

1921

- First report on the use of bacteriophage in human
- 6 patients
- Anthrax and furoncles
- Subcutaneous injections of Staphylococcus bacteriophages
- Various doses
- Complete recovery of those lesions within 24-48 hours
- Side effect: fever (for some patients) and local pain

Bruynoghe R, Maisin J. C Soc Biol.1921

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 de l'addition du 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 acmés 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 Soc Biol.1921

PHAGOTHERAPY

PRO:

- Active against Gram +, Gram -, and MDR bacteria
- Specific for a single bacteria
- Narrow antibacterial spectrum
- Few if any side effects
- Wide distribution upon systemic administration
- Modulation of the inflammatory response
- Costs
- Increased efficacy as compared to antibiotics

PHAGOTHERAPY

CON:

- Specific for a single bacteria (that has to be cultured)
- Narrow antibacterial spectrum
- Dose ? Route of administration ? Treatment duration ?
- Lytic phages (Temperate phages ?)
- Virion solution stability ?
- Transfer of genetic material from a bacteria to another
- ORFan genes
- Bacterial lysis related LPS release
- Resistance mechanisms
- Public health insurance do not cover phagotherapy
- FDA and EMA

PHAGOTHERAPY

- RANDOMIZED CONTROLLED TRIALS
- OTHER REPORTS
- (MY) EXPECTATIONS

HUMAN RANDOMIZED TRIALS

Babalova et al	Bacterial dysentry directed prophylactic treatment in children	Zh Mikrobiol Epidemiol Immunobiol. 1968
Carlton R.	PK and safety of an IV phage solution against VRE in healthy volunteers (Ia)	Personal communication
Bruttin et al	Oral T4 phages directed against E.coli in 15 healthy volunteers	Antimicrob Agents Chemother. 2005
Wright et al	AB resistant Pseudomonas directed phages in patients with chronic otitis	Clin Otolaryngol. 2009
Sarker et al	Impact of oral T4 phage cocktail on fecal microbiota in healthy volunteers	Virology. 2012
Brüssow et al	Safety of oral t4-like phage cocktails in children with E.coli diarrhea	Virology. 2012
McCallin et al	Safety of oral Microgenin® 5 adults and 5 10y-old children	Virology. 2013

JAMA®
The Journal of the American Medical Association

Bacteriophage Therapy in Infective Childhood Asthma

Heinz J. Wittig, MD, Joseph F. Raffetto, MD, and Richard Bason, MD

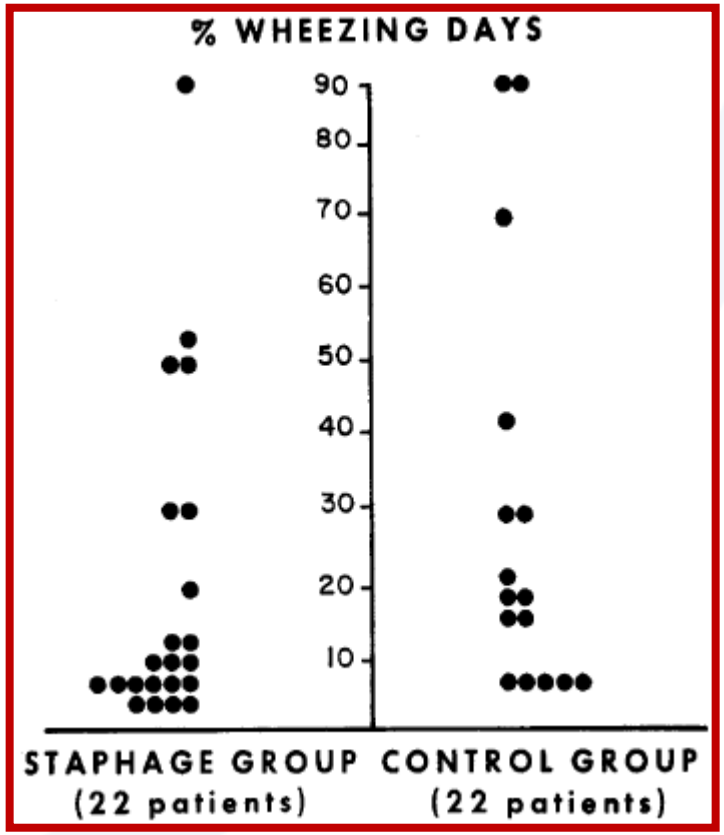
44 children – « infective asthma »
Randomization: 4 groups

2 groups received weekly injection of « staphylococci lysate + 10 billions active phages »

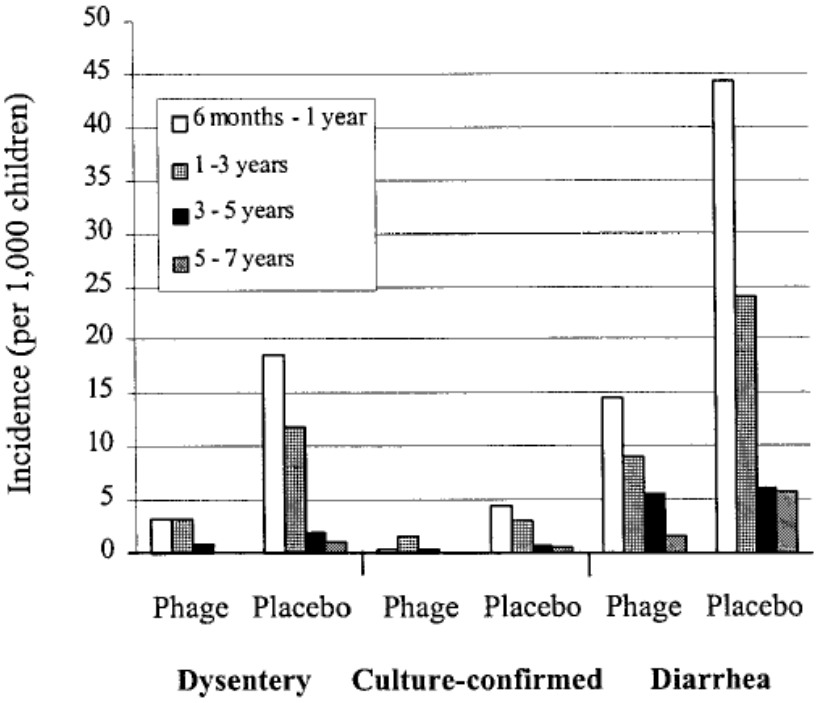
Significant decrease in wheezing days

No difference on patient scattergram

No difference in the occurrence of positive staphylococcal culture.



Wittig HJ et al. JAMA.1966; 196(5): 435

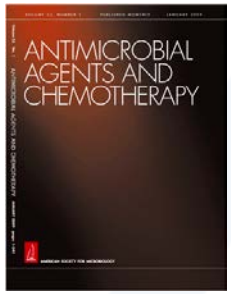


- 30769 children – 6M-7y
- 1963-64
- Phages against bacterial dysentery
- Shigella phages
- 1 oral dose every 7 days
- Versus placebo

Clinical: 3.8 fold higher dysentery in the placebo group

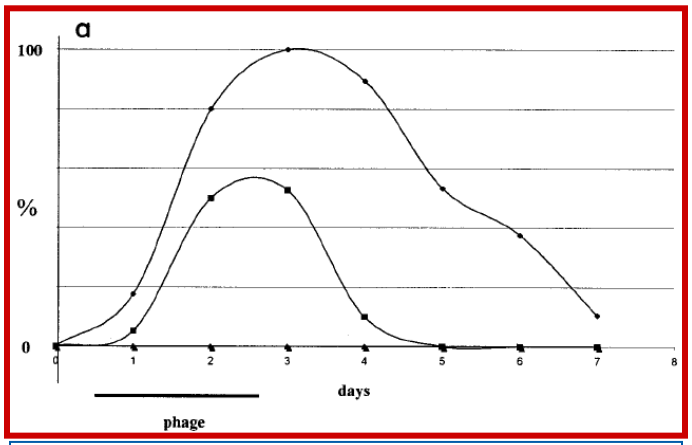
Microbiological: 2.8 fold higher

Babalova EG et al. Zh Mikrobiol Epidemiol Immunobiol.1968

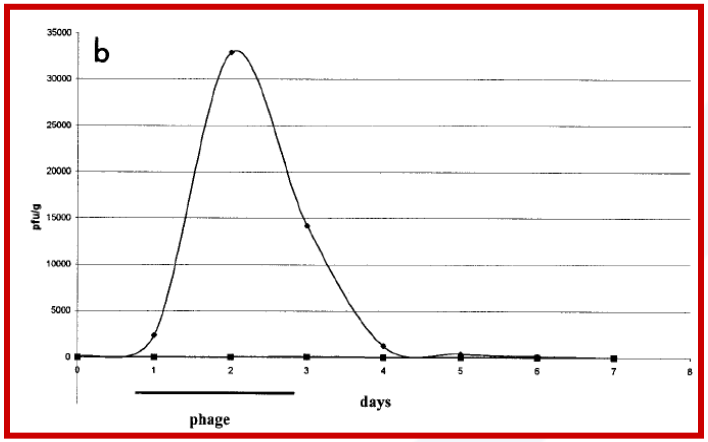


Human Volunteers Receiving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy

- 15 human volunteers / Nestlé Research Center - Lausanne
- T4 phages specific for E coli
- Placebo / Low dose (10^3 PFU/ml) / High dose (10^5 PFU/ml) – 3t/d for 2 days



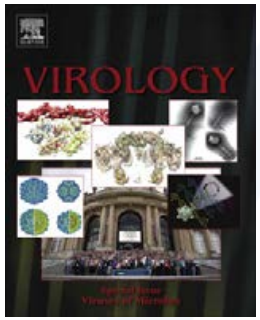
Prevalence of phage positive stools samples



Mean phage stool titer

- 5 mild AE: gastric pain, nausea, increased persitaltism, 1 sore throat
- No detection in blood, no immune response

Bruttin et al. AAC.2005



Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh

- 15 human volunteers
- T4 phages cocktail (lytic phages)
- Placebo / Low dose (10^7 PFU/ml) / High dose (10^9 PFU/ml)
- The 3 treatments in random order – 2 days treatment

- Phages were detected in 64%, 30%, 28% of stools samples respectively
- 1% of the orally administered phages recovered in the stools
- No side effects
- No impact on the fecal microbiota

Sarker SA et al. Virology.2012; 434:222-32

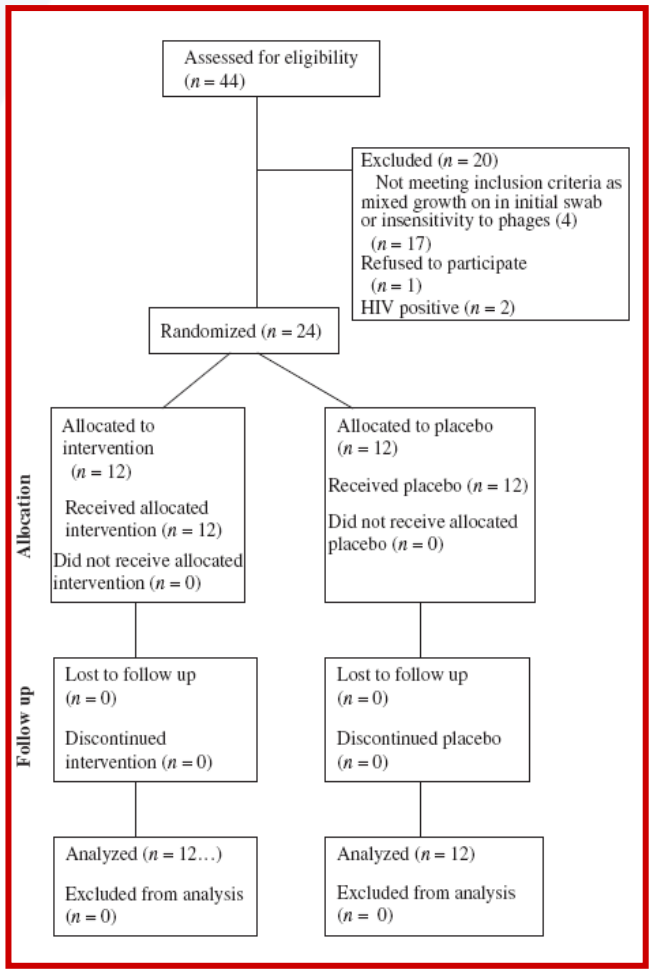
Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial

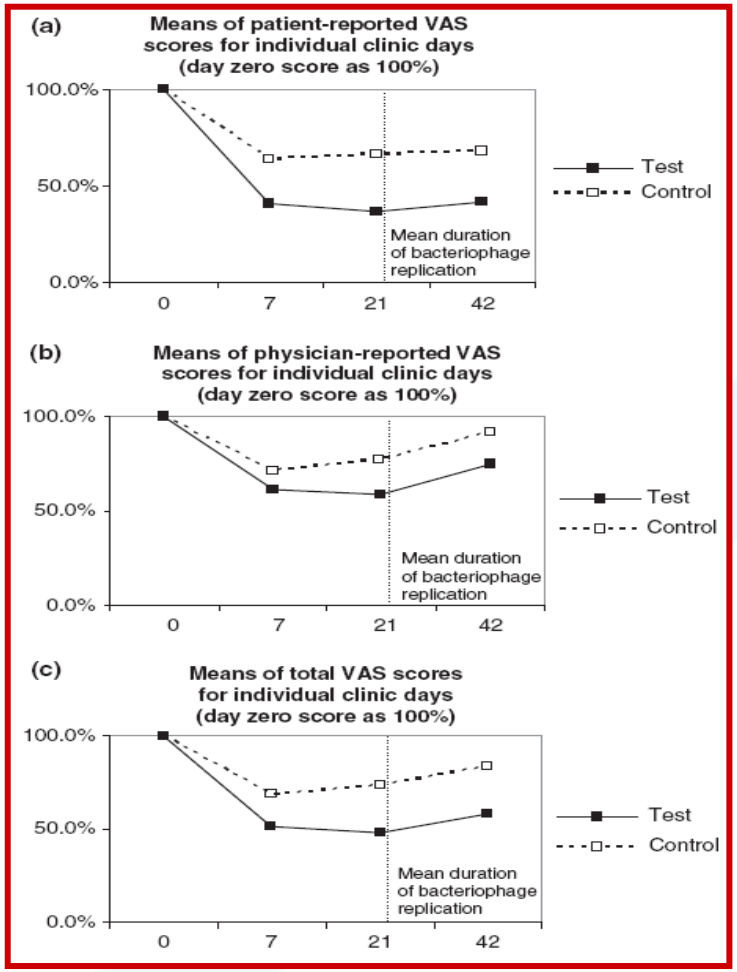
NCT00663091

- Wound Care Center, Lubbock, Texas
- 42 patients with chronic venous leg ulcer (> 30 days duration)
- Phase I, prospective, double blinded study
- Cocktail phages directed against:
  - E. coli,
  - Staphylococcus aureus, (WPP-201 – Intralytix).
  - Pseudomonas aeruginosa
- No prior sensitivity study
- No difference in cure rate (interference with the dressing ?)
- No adverse reaction

Rhoads et al. J Wound Care.2009

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





Wright et al. Clin Otolaryngol.2009

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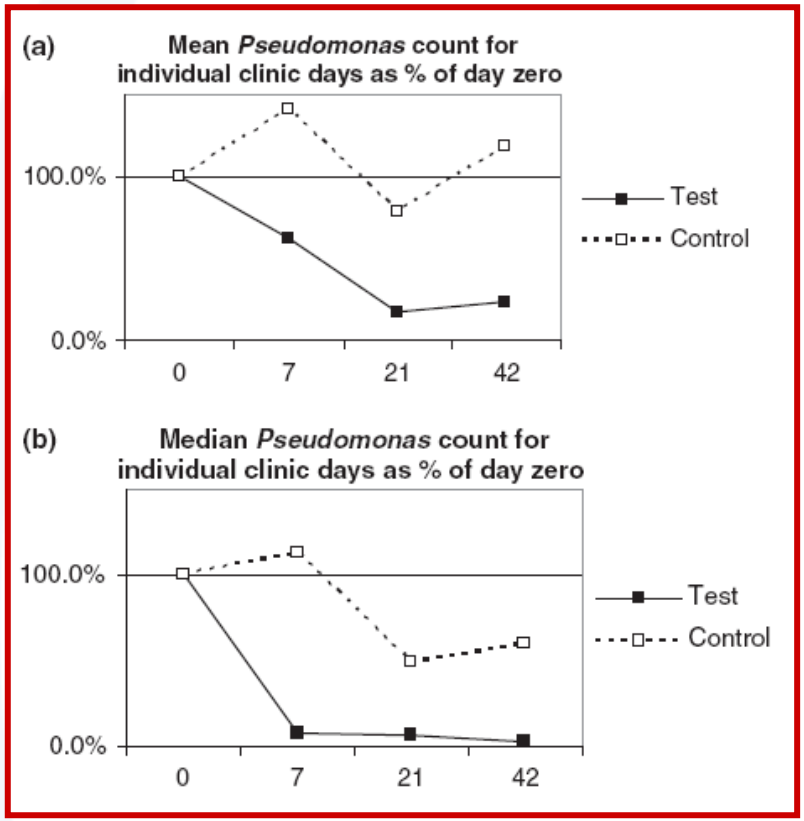
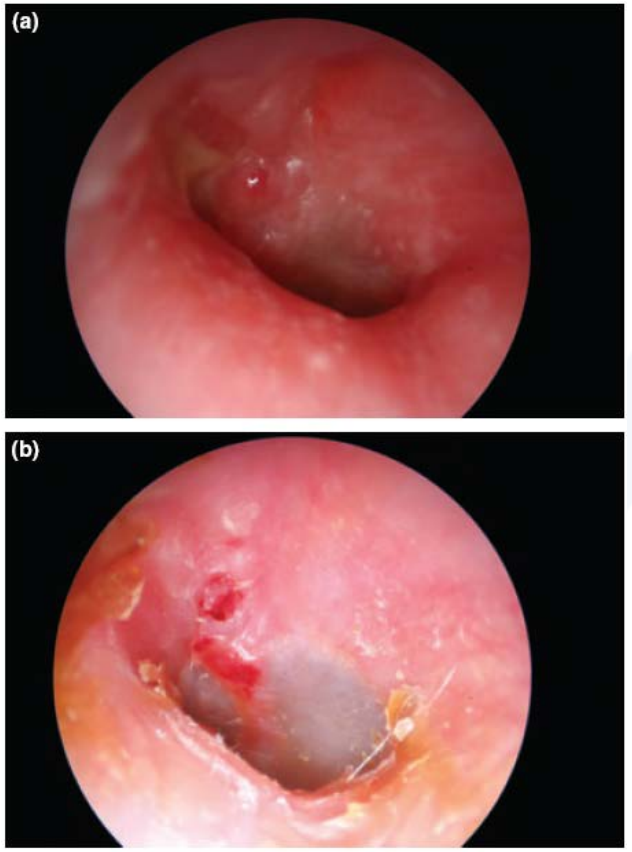




Wright et al. Clin Otolaryngol.2009

BURNS

Case report

Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients

J.A. Sivera Marza [a], J.S. Soothill [b,*], P. Boydell [c], T.A. Collyns [d]

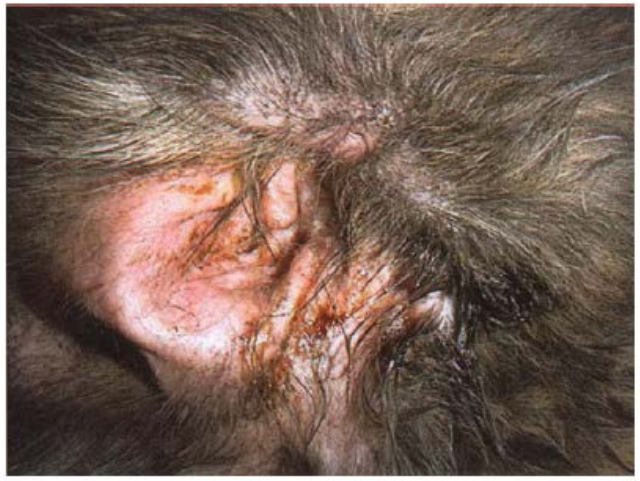


Fig. 1. Dog's ear (left) immediately before phage therapy.

400 PFU in
0,2ml saline

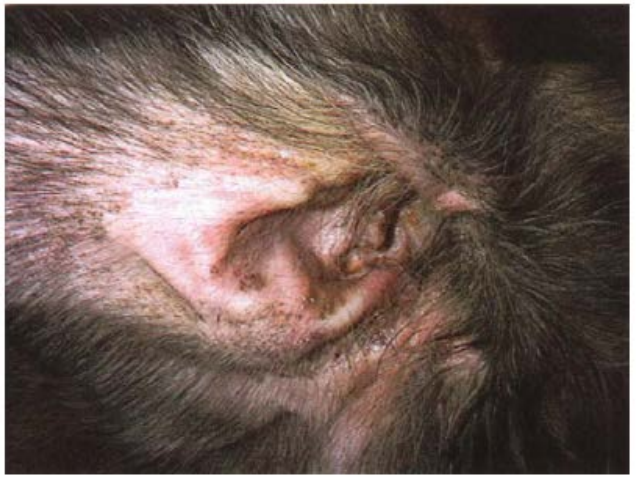


Fig. 2. Dog's ear (right) 27 h after phage therapy.

x 10^6

1,6 x 10^8 PFU
in 0,032g ear detritus

Sivera Marza et al. Burns.2006

HUMAN REPORTS

- Western experience
- Russian experience
- Georgian experience
- Polish experience
- Belgian experience

WESTERN CLINICAL DATA

- **FRANCE :**
  - Phagotehrapy used until the early 90's
  - Various type of infections (skin, orthopaedic, septicemia, gastro-intestinal, …)
  - Afterwards: some physicians obtained phages from the Eliava institute
- **USA :**
  - 1920's – 1930's
  - Eaton, Bayton Jones – 1934
    Krueger, Scribner – 1941
  - Staphylococcus aureus – Typhoid fever

Abedon ST et al. Bacteriophage.2011; 1(2):66-85

THE RUSSIAN EXPERIENCE

- Use of phages started in the 1920's
  Civilian surgical applications started in the 1930's
- The George Eliava Institute – Tbilissi – Georgia
  Numerous other Phage Centers accross the USSR
- Many animal studies
- Political – Linguistic – Cultural barriers
- Microgen Pharmaceuticals
  Microworld Ltd

THE RUSSIAN EXPERIENCE

- Dermatology – Beridze – 1938
- Ophtalmology – Rodigina – 1938
- Urology – Tsulukidze – 1938
- Stomatology – Ruchko and Tretyak – 1936
- Pediatrics – Alexandrova – 1935
  Lurie – 1938
- Otolaryngology – Emolieva – 1939
- Surgery – Tsulukidze – 1940 and 1941

Chanisvili N. Adv Virus Res.2012

THE GEORGIAN EXPERIENCE

- George Eliava (arrested and executed in 1937)

- The Eliava Institute of Bacteriophages, Microbiology and Virology
- The Institute of Microbiology Epidemiology and Bacteriophages of the All Union Ministry of Health
  Tbilissi – Georgia
  1980's: 1200 people (75% production facilities)
  up to 2 tons produced / week (liquid phage preparation)
  1990's: Lost its financial support when Georgoa left the USSR

- Isolation and characterization of phages against clinical isolates from all corners of the USSR

- Performed IV phages studies

- Intestiphage® / Pyophage®

- Cystic Fibrosis National Center in Tbilissi

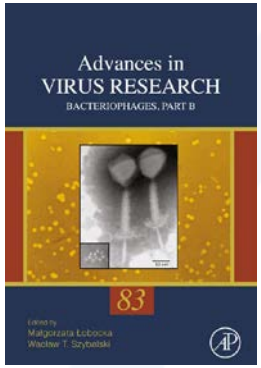
THE POLISH EXPERIENCE

- 1st Publication : 1923
- Treatment of civilians during WWII
- 1952: The Ludwig Hirzsfeld Institute of Immunology and Experimental Therapy (Wroclaw)
  - Production of phages for various clinical centers accross Poland
- > 2000 patients reported in the literature

Slopek et al. 1987
Weber-Dabrowska et al. 2000
Weber-Dabrowska et al. 2003

- 2005: 1st Outpatient Phage Unit

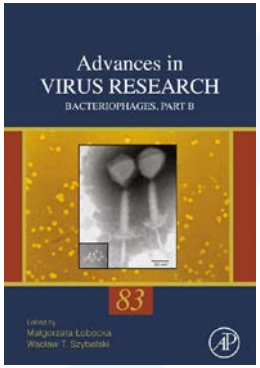
	Slopek (1987)	Weber-Dabrowska (2000)	Weber-Dabrowska (2003)
Patients	550 patients 1981-1986	1307 1987-1999	94
Type of Infections	Various Including septicemia	Suppurative AB resistant infections including septicemia	AB-resistant strains induced infections
Age	1week- 86y-old		
Combined treatment?	72,4% use of phage as stand-alone therapy		71 patients : + AB 23 patients: phage alone
Administration	Oral and local administration	Oral (and local) 1 – 12 weeks	Median:29 days
Efficacy	75-100% according to the type infection	+ : 85,9% Failure : 3,8 % Transient improvement	85,1% success



Clinical Aspects of Phage Therapy

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 ($n = 1$)

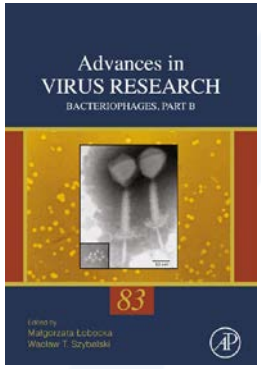
Miedzybrodzki et al. Adv Virus Res.2012



Clinical Aspects of Phage Therapy

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: $n = 49$

Miedzybrodzki et al. Adv Virus Res.2012



Clinical Aspects of Phage Therapy

	General evaluation[b] (n = 153)		Type of infection				Use of antibacterials			
			Monoinfection (n = 123)		Polyinfection[c] (n = 30)		No antibacterials (n = 109)		Antibacterial[d] used during PT (n = 44)	
Category of response to treatment[a]	n	%	n	%	n	%	n	%	n	%
A - pathogen eradication and/or recovery	28	18.3	22	17.9	6	20.0	22	20.2	6	13.6
B - good clinical result	13	8.5	11	8.9	2	6.7	7	6.4	6	13.6
C - clinical improvement	20	13.1	14	11.4	6	20.0	15	13.8	5	11.4
D - questionable clinical improvement	10	6.5	8	6.5	2	6.7	8	7.3	2	4.6
E - transient clinical improvement	33	21.6	27	22.0	6	20.0	22	20.2	11	25.0
F - no response to treatment	39	25.5	32	26.0	7	23.3	28	25.7	11	25.0
G - clinical deterioration	10	6.5	9	7.3	1	3.3	7	6.4	3	6.8
Good response (total A–C):	**61**	**39.9**	**47**	**38.2**	**14**	**46.7**	**44**	**40.4**	**17**	**38.6**
Inadequate response (total D–G):	**92**	**60.1**	**76**	**61.8**	**16**	**53.3**	**65**	**59.6**	**27**	**61.4**

Miedzybrodzki et al. Adv Virus Res.2012

- **653 patients :**

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

  - 130 patients : Phages alone
    215 patients: Phages + AB
    308 patients: No phages

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

- **653 patients :**

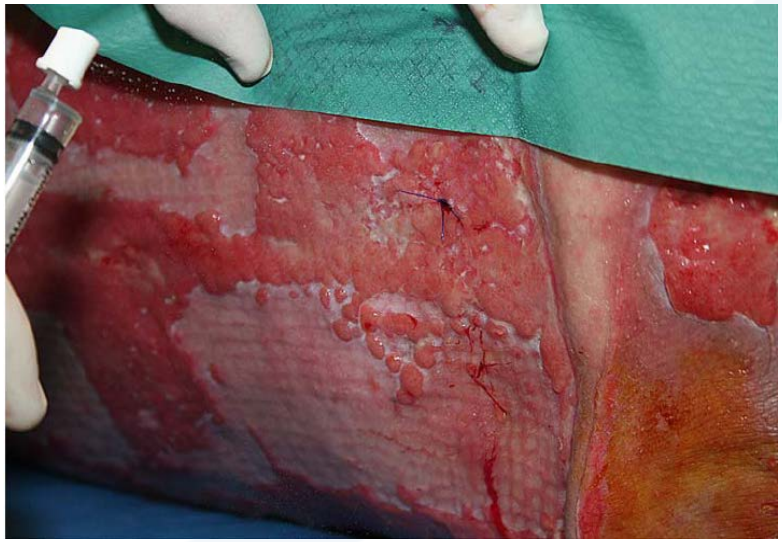
  - Various type of infections
  - Various route of administration, including IV and Intra-Arterial in case of traumatic osteomyelitis or lung disease.
  - Phage cocktail which includes Sb-1 (against Staph. Aureus)

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

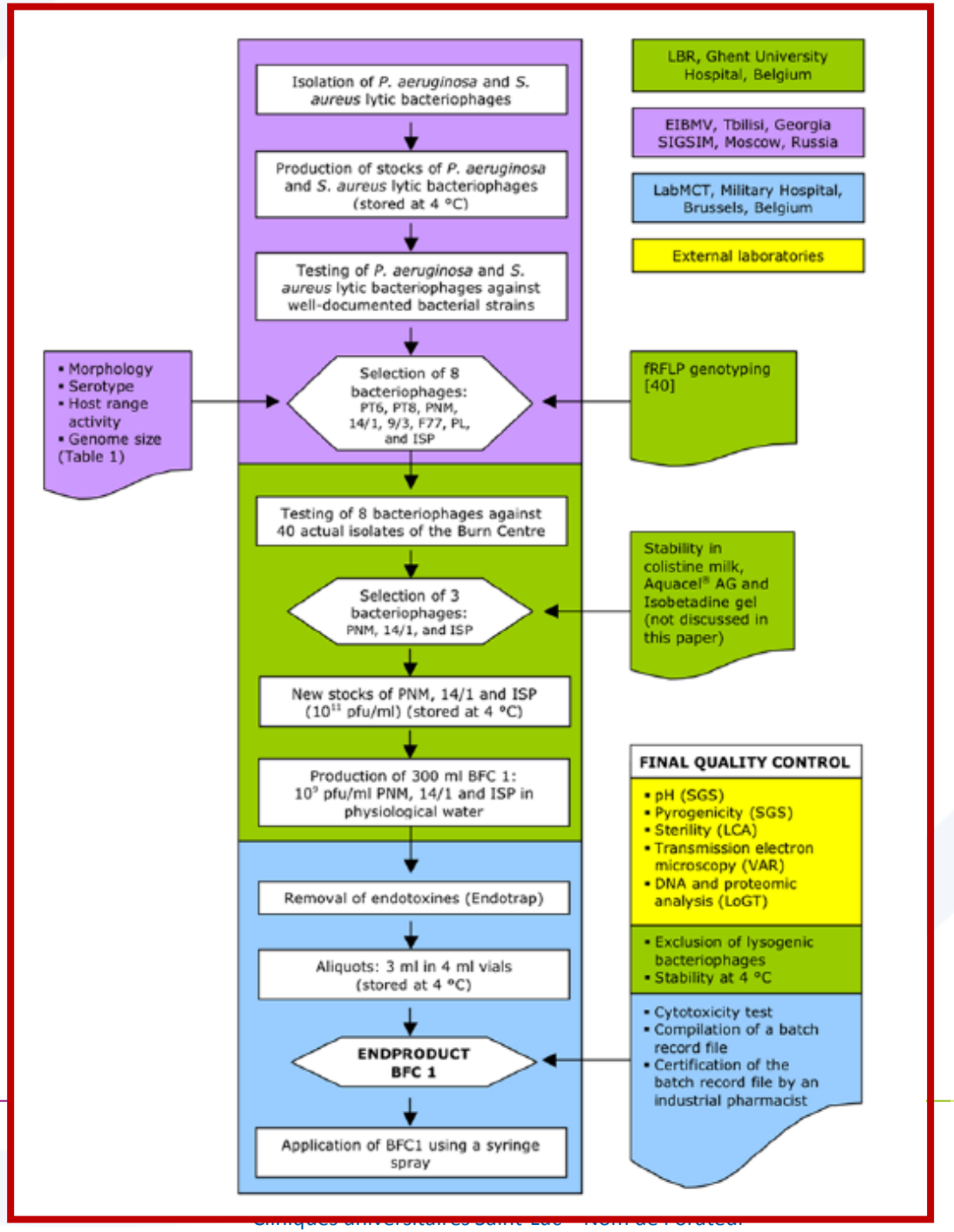
Kutter E et al. Clinical Phage Therapy. In Phage Therapy. Horizonpress.2014

The Belgian Experience: Focus on Burn Infections

- Collaboration: Belgian surgeons and scientists + Phage biologists in Moskow and Tbilissi
- Phages for Human Applications Group Europe (www.p-h-a-g-e.org)
- Burn center – Importance of Pseudomonas infections *(Pirnay et al.2003)*



Kutter E et al. Curr Pharmac Biotechnol.2010


Isolation of P. aeruginosa and S. aureus lytic bacteriophages
Production of stocks of P. aeruginosa and S. aureus lytic bacteriophages (stored at 4 °C)
Testing of P. aeruginosa and S. aureus lytic bacteriophages against well-documented bacterial strains
Selection of 8 bacteriophages: PT6, PT8, PNM, 14/1, 9/3, F77, PL, and ISP
Testing of 8 bacteriophages against 40 actual isolates of the Burn Centre
Selection of 3 bacteriophages: PNM, 14/1, and ISP
New stocks of PNM, 14/1 and ISP (10^{11} pfu/ml) (stored at 4 °C)
Production of 300 ml BFC 1: 10^{9} pfu/ml PNM, 14/1 and ISP in physiological water
Removal of endotoxines (Endotrap)
Aliquots: 3 ml in 4 ml vials (stored at 4 °C)
ENDPRODUCT BFC 1
Application of BFC1 using a syringe spray
LBR, Ghent University Hospital, Belgium
EIBMV, Tbilisi, Georgia SIGSIM, Moscow, Russia
LabMCT, Military Hospital, Brussels, Belgium
External laboratories
• Morphology
• Serotype
• Host range activity
• Genome size (Table 1)
fRFLP genotyping [40]
Stability in colistine milk, Aquacel® AG and Isobetadine gel (not discussed in this paper)
FINAL QUALITY CONTROL
• pH (SGS)
• Pyrogenicity (SGS)
• Sterility (LCA)
• Transmission electron microscopy (VAR)
• DNA and proteomic analysis (LoGT)
• Exclusion of lysogenic bacteriophages
• Stability at 4 °C
• Cytotoxicity test
• Compilation of a batch record file
• Certification of the batch record file by an industrial pharmacist

Original Article

Experimental phage therapy of burn wound infection: difficult first steps

- Main objective: evaluate the effects of a phage cocktail (BFC-1) in patients with burn wound infections due to antibiotic-resistant strains of S.aureus and P.aeruginosa
- BFC-1: 2 P.aeruginosa phages (14/1 and PNM) and 1 S.aureus phage (ISP)
- Local administration of a solution in which phages are suspended
- 9 patients / 10 applications
- Biopsy / Phage application vs usual care // 3 hours / Biopsy
- Results
  - No change in bacterial loads but …
  - No side effects

Rose T et al. Int J Burn.2014; 4(2):66-73

PHAGE ADMINISTRATION

- Oral
- Rectal
- Parenteral (including intra-venous)
- Topical
  - Intra-pleural
  - Bladder irriguation
  - Direct administration to wounds

Sprays, aerosols, mouthwash, suppositoires, bandages, eye drops, tampons.

Deresinski S. Clin Infect Dis.2009; 48:1096-1101

PHAGOTHERAPY

MY EXPECTATIONS

- MULTI DRUG RESISTANT BACTERIA
- VENTILATOR ASSOCIATED PNEUMONIA
- HAEMATOLOGIC PATIENTS with FEBRILE NEUTROPENIA
- CHRONIC INFECTIONS (Bone …) - BIOFILMS

Antibiotic-Resistant Bugs in the 21st Century — A Clinical Super-Challenge

Cesar A. Arias, M.D., Ph.D., and Barbara E. Murray, M.D.

Impact of carbapenem resistance on clinical and economic outcomes among patients with *Acinetobacter baumannii* infection in Colombia

E. V. Lemos[1,2], F. P. de la Hoz[1], N. Alvis[3], T. R. Einarson[4], E. Quevedo[2], C. Castañeda[1,2], Y. Leon[5], C. Amado[6], O. Cañon[7] and K. Kawai[8]

Burden of meticillin-resistant *Staphylococcus aureus* infections at a Swiss University hospital: excess length of stay and costs☆

M. Macedo-Viñas[a], G. De Angelis[b], P. Rohner[c], E. Safran[c], A. Stewardson[d], C. Fankhauser[d], J. Schrenzel[a,e], D. Pittet[d], S. Harbarth[a,d,*]

Antimicrobial resistance: action to combat the rising microbial challenges

Niki I. Paphitou*

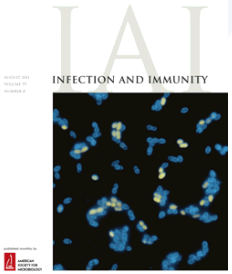
Critical shortage of new antibiotics in development against multidrug-resistant bacteria—Time to react is now☆

Laura Freire-Moran[a], Bo Aronsson[a], Chris Manz[b], Inge C. Gyssens[c,d], Anthony D. So[b], Dominique L. Monnet[e], Otto Cars[f,g,*], the ECDC-EMA Working Group[1]

Current Opinion in Microbiology

The antibiotic R&D pipeline: an update

Daniela Jabes

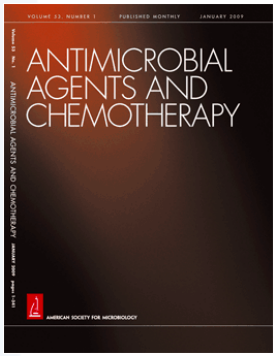


Bacteriophage Therapy Rescues Mice Bacteremic from a Clinical Isolate of Vancomycin-Resistant *Enterococcus faecium*

Biswajit Biswas,[1,2] Sankar Adhya,[1] Paul Washart,[1,2] Brian Paul,[1] Andrei N. Trostel,[2] Bradford Powell,[1,2] Richard Carlton,[2] and Carl R. Merril[1]*

National Institutes of Health, Bethesda, Maryland 20892,[1] and Exponential Biotherapies, Inc., Pt. Washington, New York 11050[2]

Biswas et al. Infect Immun.2002

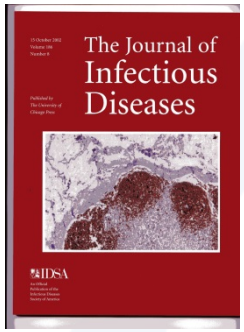


Experimental Phage Therapy in Treating *Klebsiella pneumoniae*-Mediated Liver Abscesses and Bacteremia in Mice[▽]

Chih-Hsin Hung,[1]† Chih-Feng Kuo,[2]† Chiou-Huey Wang,[3] Ching-Ming Wu,[4] and Nina Tsao[5]*

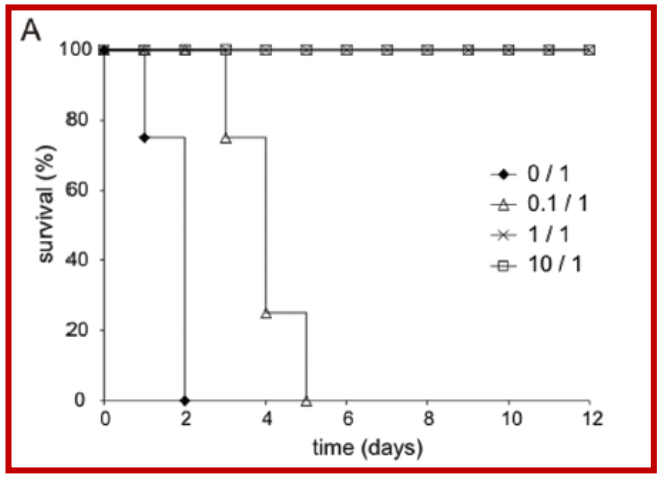
Institute of Biotechnology and Chemical Engineering[1] and Departments of Nursing[2] and Biological Science and Technology,[5] I-Shou University, Kaohsiung City, Department of Cell Biology and Anatomy, College of Medicine, National Cheng Kung University, Tainan,[4] and Department of Clinical Pathology, E-DA Hospital, Kaohsiung City,[3] Taiwan

Hung CH et al. Antimicrob Agents Chemother.2011

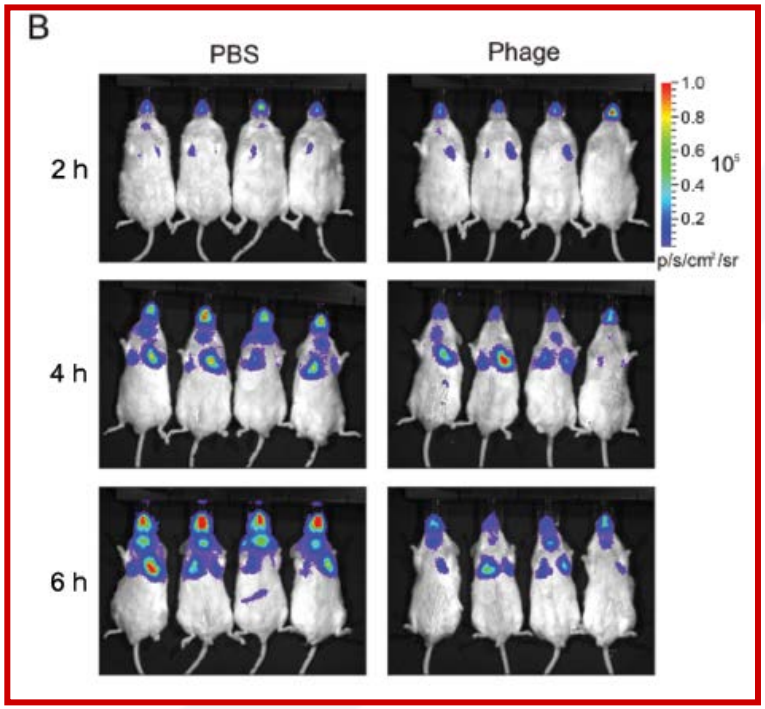


Bacteriophages Can Treat and Prevent *Pseudomonas aeruginosa* Lung Infections

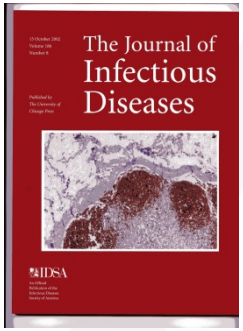
Intranasal inoculation of 10^7 pseudomonas



Treatment with PBS or
various phage concentrations



Debarbieux et al. JID.2010



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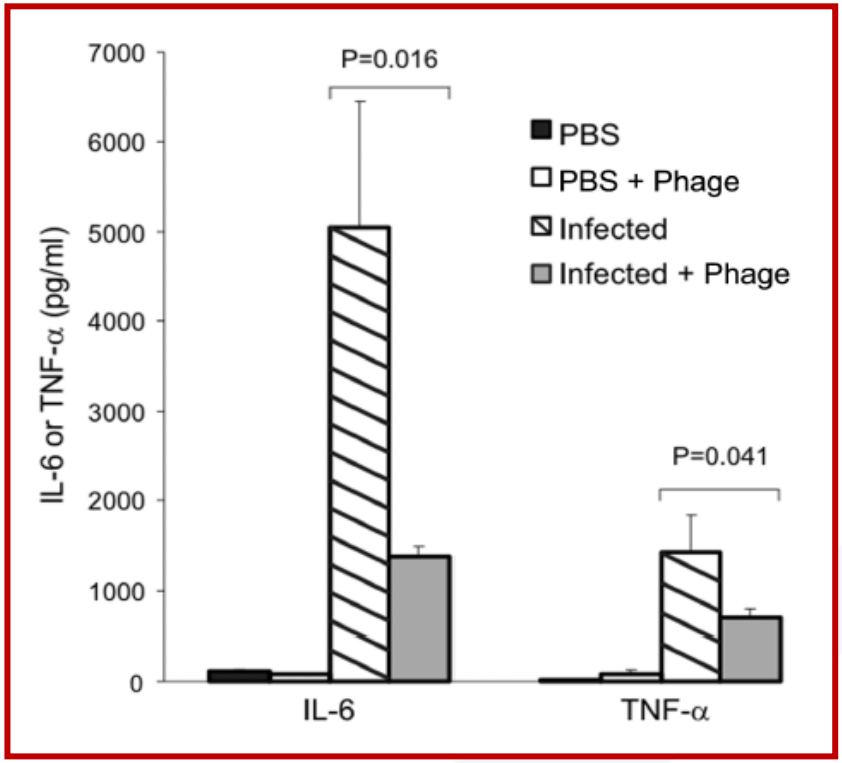
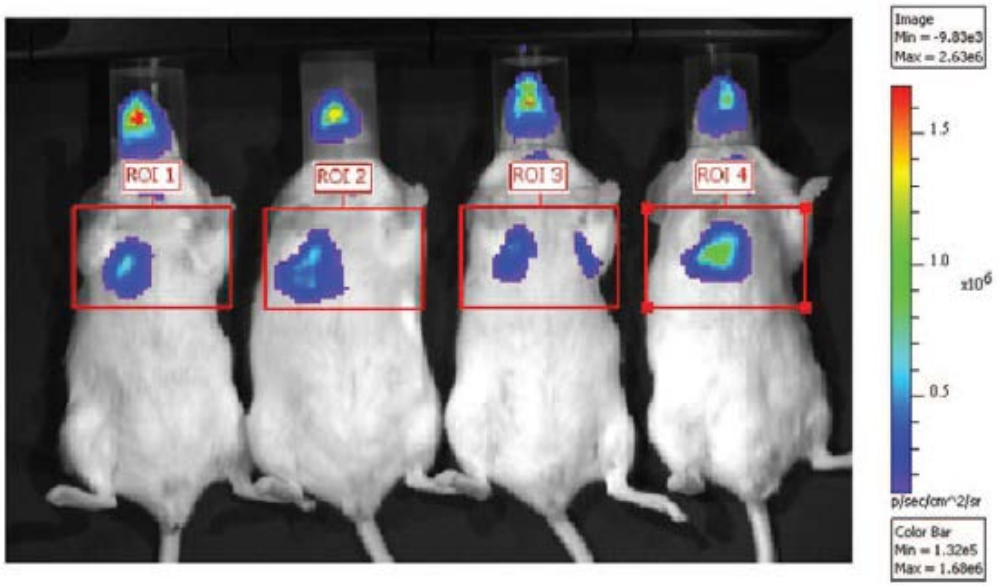
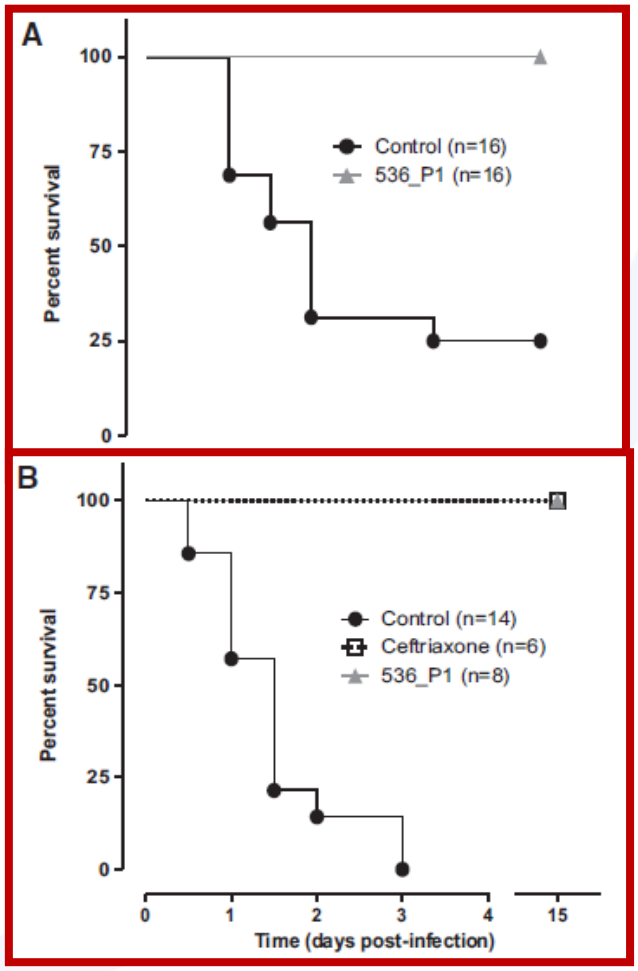


Debarbieux et al. JID.2010

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



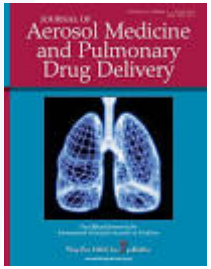


Dufour et al. Crit Care Med. 2015; In press

Research paper

Feasibility of spray drying bacteriophages into respirable powders to combat pulmonary bacterial infections

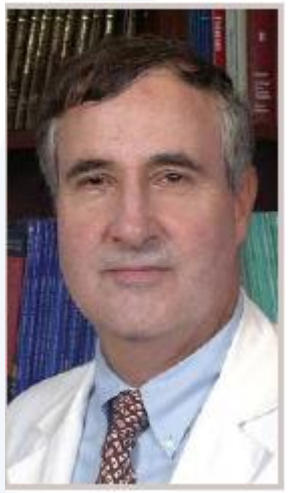
Vandenheuvel D et al. Eur J Pharamac Biopharmac.2013



Bacteriophage Delivery by Nebulization and Efficacy Against Phenotypically Diverse *Pseudomonas aeruginosa* from Cystic Fibrosis Patients

Sahota JS et al. J Aerosol Med Pulm Drug Deliver.2015; in press

NEUTROPENIC PATIENTS



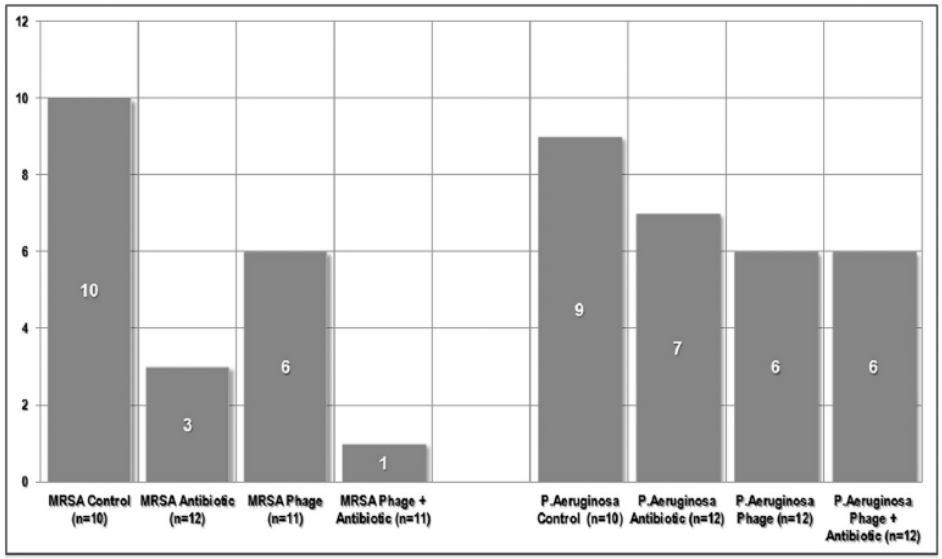
Steven M. Opal
Professor of Medicine
Alpert Medical School of Brown University
Chief Infectious Disease Division
Pawtucket, RI

- Neutropenic rat model orally colonized with Pseudomonas
- Various doses of phages directed against the Ps strain given orally after laboratory evidence of Ps colonization of gut mucosa by serial fecal cultures
- Effect on mortality and bacterial loads

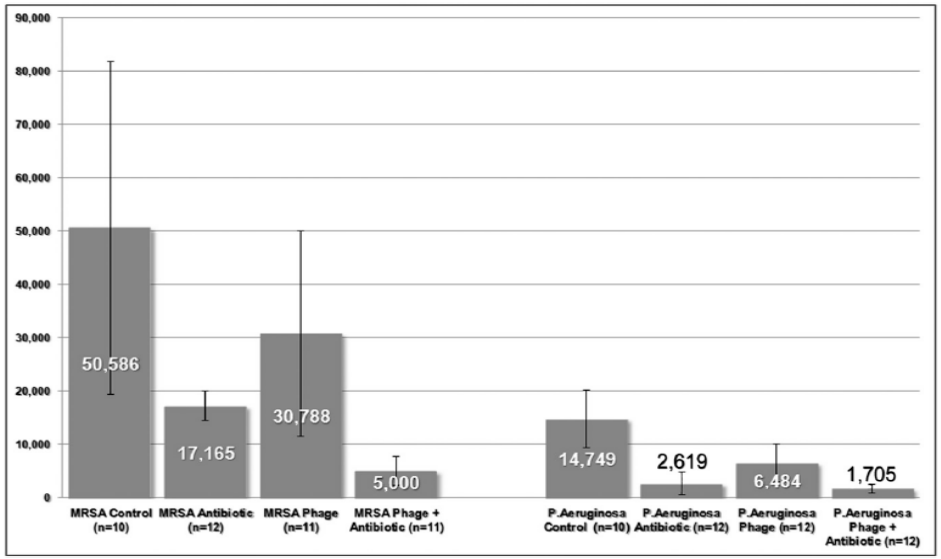
Bacteriophage Therapy in Implant-Related Infections

An Experimental Study

Model of tibial infection in rats
MRSA and Pseudomonas human infection strains



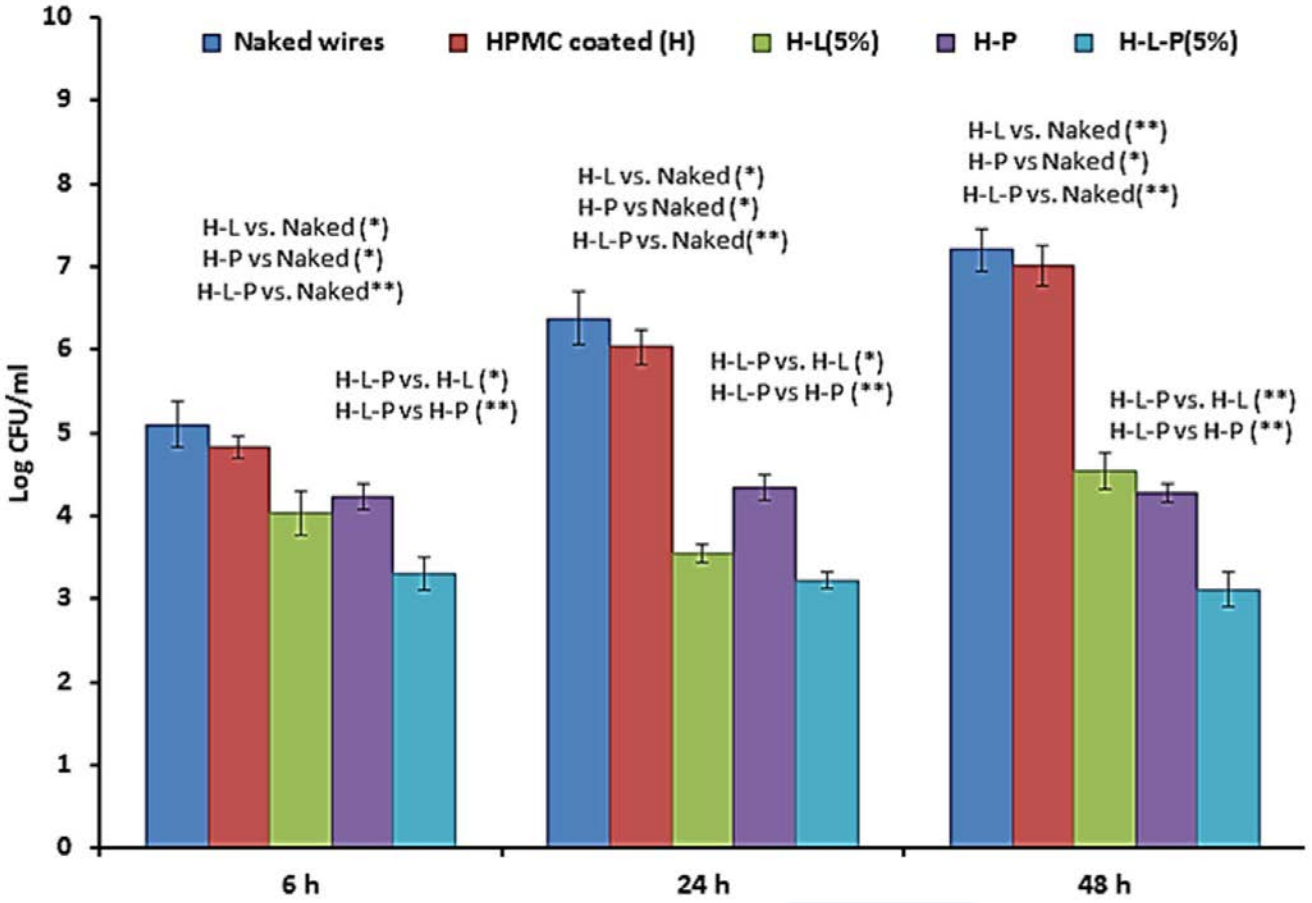
Number of subjects with positive gram staining



Quantitative culture counts

Ylmaz C et al. J Bone Joint Surg Am.2013

Bacteriophage Mediated Killing of *Staphylococcus aureus* In Vitro on Orthopaedic K Wires in Presence of Linezolid Prevents Implant Colonization



Kaur S et al. PLoS One.2014

Bacteriophages and Phage-Derived Proteins – Application Approaches

Drulis-Kawa et al. Curr Med Chem.2015; 22:1757-73

Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria

Yosef et al. PNAS.2015

ONGOING PROJECTS

- The PHAGOBURN Project (NCT02116010)
- Belgian Royal Military Hospital Projects
- Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences (NCT00945087) (12/2005)

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

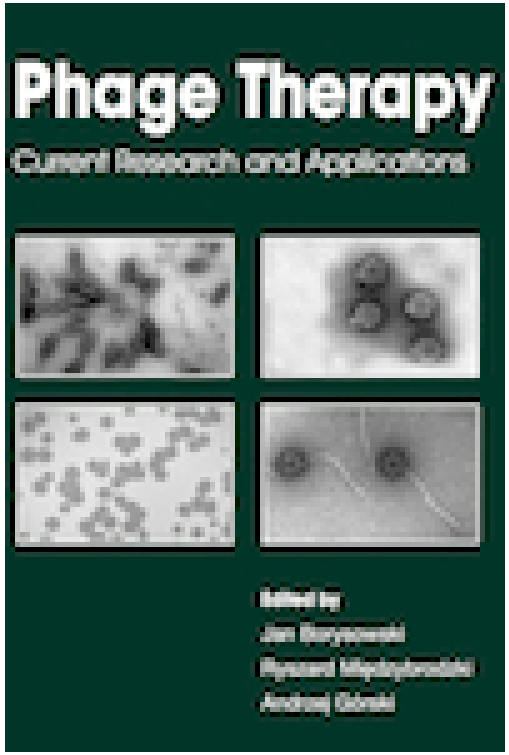
Queen Astrid Military Hospital Brussels

- Placebo controlled multicenter clinical trial
- Nasal / Throat decontamination of S. aureus or P. aeruginosa
- ICU patients
- 40 patients are intended to be enrolled

Gravitz L. Nature.2012

PHAGE APPLICATIONS

- Treatment of biofilms
- Veterinary applications
- Defence in biothreats
- Control of Pathogens in food systems
- Phages proteins (lysins …)
- Phage as delivery vectors
  - Antimicrobials
  - Vaccines


Phage Therapy
Current Research and Applications