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Authors: Lika Leshkasheli, Mzia Kutateladze, Nana Balarjishvili, Darejan Bolkvadze, Jonathan Save, Frank Oechslin, Yok-Ai Que, Grégory Resch



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Efficacy of Newly Isolated and Highly Potent Bacteriophages in a Mouse Model of XDRAB Bacteremia

Lika Leshkasheli¹, Mzia Kutateladze¹, Nana Balarjishvili¹, Darejan Bolkvadze¹, Jonathan Save², Frank Oechslin^{2,3}, Yok-Ai Que⁴ and Grégory Resch^{2*}.

¹The Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia.

²Department of Fundamental Microbiology, University of Lausanne, Switzerland.

³current address: Department of Biochemistry, Microbiology and Bio-Informatic, University Laval, Québec, Canada.

⁴Department of Intensive Medicine, Inselspital, Bern, Switzerland.

***Corresponding author**

gregory.resch@unil.ch

University of Lausanne

Quartier UNIL-SORGE, Biophore Building

Department of Fundamental Microbiology

CH-1015 Lausanne, Switzerland

Phone: +41216925609

Fax: +41216925605

Highlights

- Bacteremia can be caused by *Acinetobacter baumannii*
- Extensively drug-resistant *A. baumannii* (XDRAB) strains have emerged
- The lack of new antimicrobials led to a renewed interest into phage therapy
- Phage therapy rescued mice with bacteremia induced by an XDRAB strain

Abstract

Objectives. Bacteremia can be caused by *Acinetobacter baumannii* with clinical manifestations ranging from transient bacteremia to septic shock. Extensively drug-resistant *A. baumannii* (XDRAB) strains producing the New Delhi metallo- β -lactamase, which confers resistance to all β -lactams including carbapenems, have emerged and infected patients suffer increased mortality, morbidity and length of hospitalization. The lack of new antimicrobials led to a renewed interest into phage therapy, the so-called forgotten cure. Accordingly, we tested new lytic bacteriophages in a *Galleria mellonella* and a mouse model of XDRAB-induced bacteremia. **Methods.** *Galleria mellonella* were challenged with 5.10^5 CFU of the XDRAB strain FER. Phages vB_AbaM_3054 and vB_AbaM_3090 were administrated alone or in combination 30 min. after bacterial challenge. Saline and imipenem were injected as controls. Mice were challenged i.p. with 6.10^7 CFU of *A. baumannii* FER. vB_AbaM_3054 and vB_AbaM_3090 were administrated i.p. alone or in combination 2 h after bacterial challenge. Saline and imipenem were injected as controls. Larvae and mice survival were followed for 7 days and compared with Log-Rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests.

Results. Phage-based treatments showed high efficacy in larvae (ca. 100% survival at 80 h) and mice (ca. 100% survival at day 7) compared to the untreated control (0% survival at 48 h and 24 h in larvae and mice, respectively).

Conclusions. The present data reporting efficacy of phage therapy in a mouse model of bacteremia support the development of phage-based drugs to manage infection due to multi-drug resistant *A. baumannii* and particularly XDRAB.

Keywords: *A. baumannii*; bacteriophage; phage therapy; bacteremia; sepsis; XDRAB; MDRAB.

1. Introduction

Despite naturally present on human skin¹, the aerobic Gram-negative bacterium *Acinetobacter baumannii* is associated with major outbreaks of nosocomial infections, especially in severely ill patients hospitalized in intensive care units (ICUs)². It produces various types of infections such as bacteremia, pneumonia, endocarditis, meningitis and skin, soft-tissues and urinary tract infections. In view of its extraordinary capacity to escape currently available antibiotics, it has been classified amongst the “ESKAPE” pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsellia pneumoniae*, *A. baumannii*, and Enterobacter species)^{3,4}. Infectious diseases specialists consider this bacterium as an emerging threat more worrying than methicillin-resistant *S. aureus* (MRSA) and a recent study involving 27 ICUs from nine European countries listed it the third most common pathogen encountered in patients admitted with or having developed nosocomial pneumonia⁵. Tigecycline, the first representative of the new glycylicycline class of antibiotics^{6,7}, showed therapeutic efficacy against multidrug resistant *A. baumannii* (MDRAB), but several reports of breakthrough infections warranted caution for its use against this pathogen⁸⁻¹⁰. Moreover, extensively drug-resistant *A. baumannii* (XDRAB) strains producing the New Delhi metallo- β -lactamase, which confers resistance to all β -lactams including last resort carbapenems, have very recently emerged^{11,12} and infected patients have increased mortality, morbidity and length of hospitalization¹³. Of major concern, there is a worrying lack of new agents with new targets or mechanisms of action against MDR Gram-negative bacteria, and only two such potential molecules were identified in early stage of development in 2009¹⁴. Therefore, the time has come for global commitment to develop new antibacterial drugs for treating MDR Gram-negative associated infections, and MDRAB associated infections in particular. Accordingly, several preventive and therapeutic strategies are considered, amongst which the promising phage therapy approach¹⁵. In the present study, we characterized two newly isolated bacteriophages against *A. baumannii* and assessed their activity *in vitro* and *in vivo* in *Galleria mellonella* and in a mouse

model of bacteremia. Their remarkable efficacy either as mono-therapy or in combination compared to imipenem in the mouse model combined with optimal *in silico* safety profiles suggest these phages could be developed as alternatives for the treatment of *A. baumannii*-invasive infections.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The OXA-23 and AmpC multidrug resistant *A. baumannii* strain FER (FER)¹⁶ kindly provided by Patrice Nordmann was chosen for the *in vivo* experiments. In addition, 82 *A. baumannii* clinical isolates collected at the University hospital of Lausanne (CHUV) were obtained from the Institute of microbiology of the University of Lausanne (IMUL) and investigated in this study (Table S1). The 83 isolates were grown in Luria Broth (LB) at 37°C and 200 rpm for 16 h and on LB agar (LA) plates aerobically at 37°C for 24 h.

2.2. Antibiotic susceptibility

The *A. baumannii* strain collection was provided with antibiograms previously determined using the Vitek-2 apparatus with AST-N420 card (Biomérieux SA, Marcy l'Etoile, France) and interpreted according to the most recent EUCAST clinical breakpoints¹⁷ (Table S1).

2.3. Isolation of bacteriophages

Phages were amplified from samples of 12.5 mL raw sewage water (Vidy wastewater treatment plant, Lausanne, Switzerland) mixed with 1.5 mL of LB 10X and 1 mL of an overnight (o/n) culture of strain FER. After 24 h at 37°C and 200 rpm, amplification mixture was centrifuged at 4000 g for 15 min. Supernatant was filtered through 0.45 µm syringe filters (Cobetter Lab, Hangzhou, China) and stored at 4°C until further use. Supernatant was further investigated through double-layer assays to obtain individual Plaque Forming Units (PFU). Briefly, 0.2 mL of bacterial o/n culture and 0.1 mL serial dilutions of supernatant were mixed in 4 mL LB-soft agar and poured on top of

LA plates. Individual PFU were picked and mixed in 5 mL LB supplemented with 250 μ L of an o/n culture of FER. After 24 h at 37°C, mixture was centrifuged at 4000 rpm for 15 min. and filter sterilized through 0.22 μ m syringe filters. The filtrate was processed through double-layer assay as described above and a second PFU was picked. The procedure was repeated at least three times to ensure selection of individual phages, which were stored at 4°C until further use.

2.4. Determination of host ranges

Each phage was tested on each *A. baumannii* strain of our collection using classical drop test assay. Briefly, a strain was poured in a layer of soft agar and 5 μ L drops of phage preparations were deposited on top of the solidified layer. After 24 h incubation at 37°C, plates were read by eye and lysis zones scored CL (Clear Lysis), SCL (Semi-Clear Lysis) or OL (Opaque Lysis). For FER, which was chosen for the *in vivo* tests, serial dilutions of stock solution were deposited in order to check for infectivity.

2.5. Phage adsorption

Exponentially growing *A. baumannii* FER (10^8 CFU/mL) were mixed with corresponding phages at MOI of 1 and incubated at 37°C in a water-bath. 100 μ L of the mixture was collected every 5 min. for 60 min. and diluted 100-time in LB supplemented with 4% (vol/vol) chloroform. For each tube, phage titer was determined through double-layer assay using *A. baumannii* FER as host strain. The rate of adsorption was calculated accordingly. Experiments were performed in triplicate.

2.6. Electron microscopy

Morphology of phage particles was studied using a JEOL 100C electron microscope (Jeol, Akishima-Shi, Tokyo, Japan). Phage suspension (10^9 - 10^{10} PFU/mL) was transferred onto carbon-coated copper grids for 30 sec. to let particles settle and stained with 1% uranyl-acetate for 40 s.

Filter paper was used to wick off excess of sample. Grids were examined at different magnifications.

2.7. Full genome sequencing and analysis

Phage genomic DNA was extracted and purified using a classical phenol/chloroform extraction method¹⁸.

2.8. Illumina Sequencing and reads assembly

vB_AbaM_3054 genomic DNA fragment sequencing libraries was prepared using the TruSeq Nano DNA LT Library Preparation Kit (Illumina; San Diego, USA) according to the supplied protocol and using 200 ng of genomic DNA. The resulting library was 100 nt paired-end sequenced on the Illumina HiSeq 2500 using TruSeq PE Cluster Kit v4 reagents and TruSeq SBS Kit v4 reagents. Sequencing data were processed using the Illumina Pipeline Software version 1.84. After adapter trimming using trimmomatic-0.36, assembly was done automatically using spades¹⁹.

2.9. PacBio sequencing and assembly

Genomic DNA of phage vB_AbaM_3090 was sequenced through Pacific Bioscience (PacBio) sequencing. The DNA was sheared in a Covaris g-TUBE (Covaris, Woburn, MA, USA) to obtain 20 kb fragments. After shearing the DNA size distribution was checked on a Fragment Analyzer (Advanced Analytical Technologies, Ames, IA, USA). 1.3 µg of the sheared DNA was used to prepare a SMRTbell library with the PacBio SMRTbell Template Prep Kit 1 (Pacific Biosciences, Menlo Park, CA, USA) according to the manufacturer's recommendations. The library was sequenced on one SMRT cell with P6/C4 chemistry and MagBeads on a PacBio RSII system (Pacific Biosciences, Menlo Park, CA, USA) at 240 min movie length. The PacBio module "RS_HGAP_Assembly.2" in SMRTpipe version v2.3.0 was used to assemble the obtained reads.

2.10. Genome annotation

Open reading Frames (ORF, minimum size of 100 amino acids) and tRNA encoding genes were identified on phage genomes and annotated using ORF Finder²⁰ and Prokka²¹. In addition, phage genomic sequences were checked for genes coding virulence factors and antibiotic resistance using the blast interface of the Virulence Factors of Pathogenic Bacteria database (www.mgc.ac.cn/VFs) and the ResFinder tool from the Center for Genomic Epidemiology (<https://cge.cbs.dtu.dk/services/ResFinder>), respectively.

2.11. In vitro turbidity assays in 96-well plates

FER was grown in 10 mL LB at 37°C and 200 rpm for 16 h. After centrifugation of the culture at RT and 4000 rpm for 20 min. the bacterial pellet was thoroughly resuspended in 50 mL saline. 10 µL of this bacterial suspension (i.e. 10⁶ CFU) were mixed in 96-well plates with 10 µL of phage suspensions at different concentrations (i.e. 10⁴, 10⁶ and 10⁸ PFU) or 10 µL of imipenem at 5 mg/mL (final concentration of 167 µg/ml) and 280 µL LB. The microtiter plate was placed in a microplate reader set at 37°C and first measurement at OD_{600nm} was taken immediately. Additional measurements were taken every 10 min for 24 h. Microplate was shaken for 3 sec. before each measurement. All experiments were performed in triplicate.

2.12. A. baumannii experimental bacteremia in Galleria mellonella

Wax moths (n = 18) were challenged in the last right pseudopod with 5.10⁵ CFU of FER in 5 µL saline. Phages (5.10⁷ PFU in 10 µL saline; MOI = 100) were administrated in the last left pseudopod alone or in combination, by a single bolus injection 30 min after bacterial challenge. Saline and imipenem at 5 mg/mL were injected as controls. The survival rates of larvae were followed over a period of 80 h and compared with Log-Rank (Mantel-Cox) and Gehan-Breslow-

Wilcoxon tests using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

2.13. A. baumannii experimental bacteremia in mice

The mice model of bacteremia was carried out in strict accordance with the recommendations of the Swiss Federal Act on Animal Protection. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Consumer and Veterinary Affairs Department of the State of Vaud (Permit N°3065). A total of 30 CD1 Swiss female mice (Charles River Laboratories, L'Arbresle, France), with an average weight of 21 ± 1 g, were used in this study. The animal sample size (n) was estimated to be 6 with the formula for dichotomous variables (expected $p_c = 1$ and $p_e = 0.2$; with p_c = death event in control group, p_e = death event in experimental group and $C=7.85$)²². In order to induce bacteremia, the mice were challenged i.p. with $6 \cdot 10^7$ CFU of FER in 100 μ L saline. Bacteremia state at the time of the initial treatment injection was validated after aseptically removal of the left kidney and spleen from three mice 2 h after i.p injection of $6 \cdot 10^7$ CFU of FER in 100 μ L saline. Organs were homogenized in 1 ml of saline and briefly centrifuged, and supernatants were plated on blood agar plates to determine the number of viable organisms in tissues. Phages ($6 \cdot 10^9$ PFU in 200 μ L saline; MOI of 100) were administrated i.p. alone or in combination by a single bolus injection 2 h after bacterial challenge. 200 μ L saline and 200 μ L imipenem at 5 mg/mL (ca. 50 mg/kg) were injected as controls. The survival rates of animals were followed over a period of 7 days and compared with Log-Rank (Mantel-Cox) and Gehan-Breslow-Wilcoxon tests using GraphPad Prism version 5.00 for Windows.

3. Results

3.1. Isolation and characterization of *A. baumannii* phages

Two phages, named vB_AbaM_3054 and vB_AbaM_3090, were successfully isolated through classical amplification from samples of wastewater. Phage vB_AbaM_3054 formed small clear plaques surrounded by halos and phage vB_AbaM_3090 formed tiny clear plaques with no halo on a lawn of FER (Fig. S1).

3.2. Phage host range and adsorption rate

The host range of both phages was first quickly screened by drop test on the collection of 83 clinical isolates (Table S2). Both phages revealed very strain specific with CL or SCL lysis on 10.8% and 14.5% of the strains tested (Table S2). In addition, since FER was chosen for the *in vivo* study, infectivity of both phages was checked by drop tests of serially diluted stock solutions (non-diluted to 10^8 dilutions). As shown in Fig. S2, both phages formed well-separated plaques on FER at high dilutions (i.e up to 10^8 and up to 10^4 for vB_AbaM_3054 and vB_AbaM_3090, respectively) confirming infectivity of both phages on FER. Examination of phage particles morphology by TEM revealed that both phages belong to the myoviridae family. The size of vB_AbaM_3054 head is 102 x 94 nm and the size of the tail is 88 x 22 nm (Figure 1A). The size of vB_AbaM_3090 head is 113 x 108 nm and the size of the tail is 130 x 13 nm (Fig. 1B). The adsorption rate was $89\pm 4\%$ and $95\pm 2\%$ in 10 minutes for vB_AbaM_3054 and vB_AbaM_3090, respectively.

3.3. Phage genome sequencing and analysis

Both phages harbored a double-stranded DNA genome successfully purified by classical phenol/chloroform extraction.

3.3.1. vB_AbaM_3054

Sequencing of the genome of vB_AbaM_3054 through Illumina 100bp paired-end sequencing yielded a draft genome consisting of 4 contigs of 16'549 bp (vB_AbaM_3054_contig1), 14'254 bp (vB_AbaM_3054_contig2), 13'184 bp (vB_AbaM_3054_contig3) and 5'456 bp (vB_AbaM_3054_contig4) for a total length of 49'443 bp. As listed in Table S3, blastn against the “nr” database identified very few homologies with already published sequences of phage vB_AbaP_B1 (Genbank accession N° MF033347.1) and phage SH-Ab 15599 (Genbank accession N° MH517022.1). A detailed analysis revealed presence of 47 ORFs of more than 100 amino acids (aa) on the draft genome, amongst which 14 matched phage proteins using blastp against the “non-redundant protein sequences (nr)” database. While two structural genes (i.e. tail fiber proteins) were identified on vB_AbaM_3054_contig1, ORFs possibly involved in replication (DNA ligase, hydrolase, DNA topoisomerase and exonuclease) were identified on the three other contigs. ORF7 on vB_AbaM_3054_contig4 encodes for a protein showing significant homology with several cell wall hydrolases and likely corresponds to the lysin of vB_AbaM_3054.

3.3.2. vB_AbaM_3090

Sequencing of vB_AbaM_3090 through PacBio technology yielded a single contig of 104'796 bp. Blastn identified vB_AbaM_phiAbaA1 (GenBank accession N°KJ628499) as a very close neighbor with 98% identity over 98% query coverage. 166 ORFs and 13 tRNA genes were identified on vB_AbaM_3090 genome. 157 ORFs were assigned ‘hypothetical proteins’ (not shown).

3.4. Minimum inhibitory concentration (MIC) and in vitro turbidity assays

MIC of imipenem was 32 mg/L for FER (not shown). The effects of the different treatments on the *in vitro* bacterial growth are presented in Fig. 2. For clarity of the graphics, only hourly measurements are reported in the figures. vB_AbaM_3054 showed very similar patterns of bacterial growth inhibition independently to the MOI (ranging from 0.01 to 100), except that the

initial bacterial growth at 1 h was progressively inhibited with full inhibition at MOI of 100 (Fig. 2A, open squares). Of note, a secondary bacterial regrowth starting at ca. 7 h was observed at all MOI (Fig. 2A). Bacterial growth inhibition by vB_AbaM_3090 followed very similar patterns at the three different MOI and the initial bacterial growth was also fully inhibited at MOI of 100 (Fig. 2B, open squares). As for vB_AbaM_3054, the secondary growth started at ca. 7 h but followed a less steep slope (Fig. 2B and Fig. 2C). In view of these first results with monophage preparations, MOI of 100 was considered for the *in vivo* models. Therefore, combination of both phages at this MOI was further tested *in vitro*. As seen in Fig. 2C, the combination of both phages at MOI of 100 was highly synergistic and fully prevented secondary growth for up to 24 h (Fig. 2C; open diamonds). Similar results were observed *in vitro* with imipenem at the high dose of 5 mg/mL (>5 times the MIC), a concentration mimicking recommended *in vivo* dosage (for convenience only reported in Fig. 2A; open circles).

3.5. Therapeutic efficacy in a *Galleria mellonella* model of *A. baumannii* infection

Efficacy of both phages at MOI of 100, either alone or in combination was further compared to a single bolus injection of the standard of care imipenem on the survival of *Galleria mellonella* challenged with ca. $5 \cdot 10^5$ CFU of *A. baumannii* FER (Fig. 3). This bacterial inoculum led to 0% survival of the wax moths at 48 h in the untreated control group. When injected 30 min. after bacterial challenge, all treatments led to significant improved survival at 80 h ($p < 0.0001$, compared to the untreated control for all treatments) and the effect was not significantly different between the treatments (100%, ca. 83%, ca. 89% and ca. 95% survival at 80 h for vB_AbaM_3054, vB_AbaM_3090, combination of both phages and imipenem, respectively. $p = 0.3222$).

3.6. Therapeutic efficacy in a mouse model of *A. baumannii* bacteremia

Three mice were challenged i.p. with 6.10^7 CFU of FER in 100 μ L saline. Animals were euthanized 2 h post-infection and organs were homogenized and tested for presence of viable bacteria. We found $>10^5$ CFU/g in the spleen and kidney (data not shown), demonstrating infection dissemination and bacteremic state of the mice at this time point. Efficacy of both phages at MOI of 100, either alone or in combination was further compared to a single bolus injection of imipenem on the survival of mice challenged i.p. with ca. 6.10^7 CFU of FER (n= 6, Fig. 4). According to the bacteremic state previously demonstrated, this bacterial inoculum led to 0% survival of the animals at day 1 in the untreated control group. Phage treatments injected 2 h post-infection led to improved survival at day 7 ($p < 0.0001$, compared to the untreated control for all phage treatments) and the effect was not significantly different between treatments (80%, 100% and 80%, for vB_AbaM_3054, vB_AbaM_3090 and the combination of both phages, respectively. $p = 0.5913$). In opposite, with ca. 17% survival at day 7, a single bolus injection of 50 mg/kg imipenem failed to rescue the mice ($p = 0.3173$ compared to the untreated control).

4. Discussion

Bacteremia is one of the most significant infections caused by *A. baumannii* with clinical manifestations ranging from transient bacteremia to septic shock with high mortality²³ and phage therapy has been documented on multiple occasion as a promising alternative to treat *A. baumannii* infections. Indeed, the therapeutic potential of anti-*A. baumannii* phages has been demonstrated since 2015 but mainly in rodent models of wound²⁴⁻²⁶ and lung²⁷⁻²⁹ infections. Interestingly, a very recent human case report demonstrated efficacy of phage therapy (as adjunct to antibiotherapy) in a patient suffering from a disseminated resistant *A. baumannii* Infection³⁰. Of note, it has also recently been shown that strategies using prophage-derived lysin or derived peptides can rescue mice from otherwise lethal *A. baumannii* induced bacteremia^{31,32}. Accordingly, we aimed at evaluating the therapeutic potential of phages in a mouse model of bacteremia. Following our effort to isolate lytic *A. baumannii* phages, we focused here more

particularly on two very potent candidates belonging to the Myoviridae family, namely vB_AbaM_3054 and vB_AbaM_3090, harboring strong *in vitro* lytic activity against the *A. baumannii* clinical strain FER, representative of the commonly encountered serotype 2. The clinical relevance of this strain has previously been shown when it was found to harbor a plasmid (pFER) carrying a *bla*_{OXA-23} gene conferring higher levels of carbapenem resistance than the one conferred by the recombinant plasmid pOXA-23 or pOXA-58¹⁶.

Full genome sequencing of both phages revealed their potential to be considered as antibacterial agents administrable to human since neither virulence nor resistance genes could have been detected³³. In addition, absence of integrase genes argues for strict lytic life style of both phages. Taken together, these data consolidated their potential safety. *In vitro* tests revealed the therapeutic potential of the two phages, which were both able to dramatically inhibit growth of FER in turbidity assays at low (0.01), medium (1) and high (100) MOI. The curve profiles were highly similar at the different MOI, except that the initial bursts of bacterial growth, which occurred at low MOIs were totally inhibited at a high MOI of 100. Of note, in all conditions, bacterial regrowth was observed after 7 h, likely due to the selection of phage-resistant bacterial variants as often observed *in vitro*. *A. baumannii* FER was confirmed to be resistant to imipenem with MIC of 32 mg/L and it was therefore without surprise that this carbapenem performed very well *in vitro* at a concentration of 167 µg/mL (i.e. >5-times the MIC of 32 mg/L) with no detectable bacterial growth over 24 h. *In vitro* results for phages at MOI of 100 and imipenem transposed to *Galleria mellonella* in which all treatments achieved also high protection with ca. 100% survival of the larvae 80 h after the bacterial challenge. Interestingly, *Galleria mellonella* successfully predicted the outcome of phage therapy in mice in which all phage-based treatments achieved ca. 100% survival rate 7 days after bacterial challenge. This observation was in agreement with previous studies reporting *Galleria mellonella* as a useful pre-screening model in frame of the evaluation of phages as antimicrobials before testing in more sophisticated mammalian models^{34,35}. However, imipenem at 5 mg/mL rescued 95% of the *Galleria mellonella* and therefore failed to predict the outcome in

mice, in which it was inactive. This discrepancy might be explained by a bioavailability of imipenem in *Galleria mellonella* similar to the test tube situation and/or differences in the immune systems in comparison to mice³⁶. Therefore, this result poses the question of *Galleria mellonella* as a relevant *in vivo* model in this setting.

Contrasting *in vitro* results (i.e. secondary growth) with high *in vivo* potencies of monophage preparations question the clinical relevance of potential phage-resistant mutants selected *in vitro*. Additional experiments are needed to fully characterize the selected mutants in order to determine whether the observed discrepancies could be explained by a high fitness cost leading to the incapacity of the mutants selected *in vitro* to survive or infect *in vivo* as recently reported by our team for *Pseudomonas aeruginosa*³⁷ and reviewed by others³⁸.

Keeping in mind all the remaining challenges and unanswered questions, we are convinced that the present data showing efficacy of phage therapy in a mouse model of XDRAB bacteremia could pave the road to the development of phage therapy to manage systemic infections due to MDRAB and XRDAB.

Declarations

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Ethical Approval: The protocol was approved by the Committee on the Ethics of Animal Experiments of the Consumer and Veterinary Affairs Department of the State of Vaud (authorization n°3065)

Competing Interests: The authors declare that they have no competing interests.

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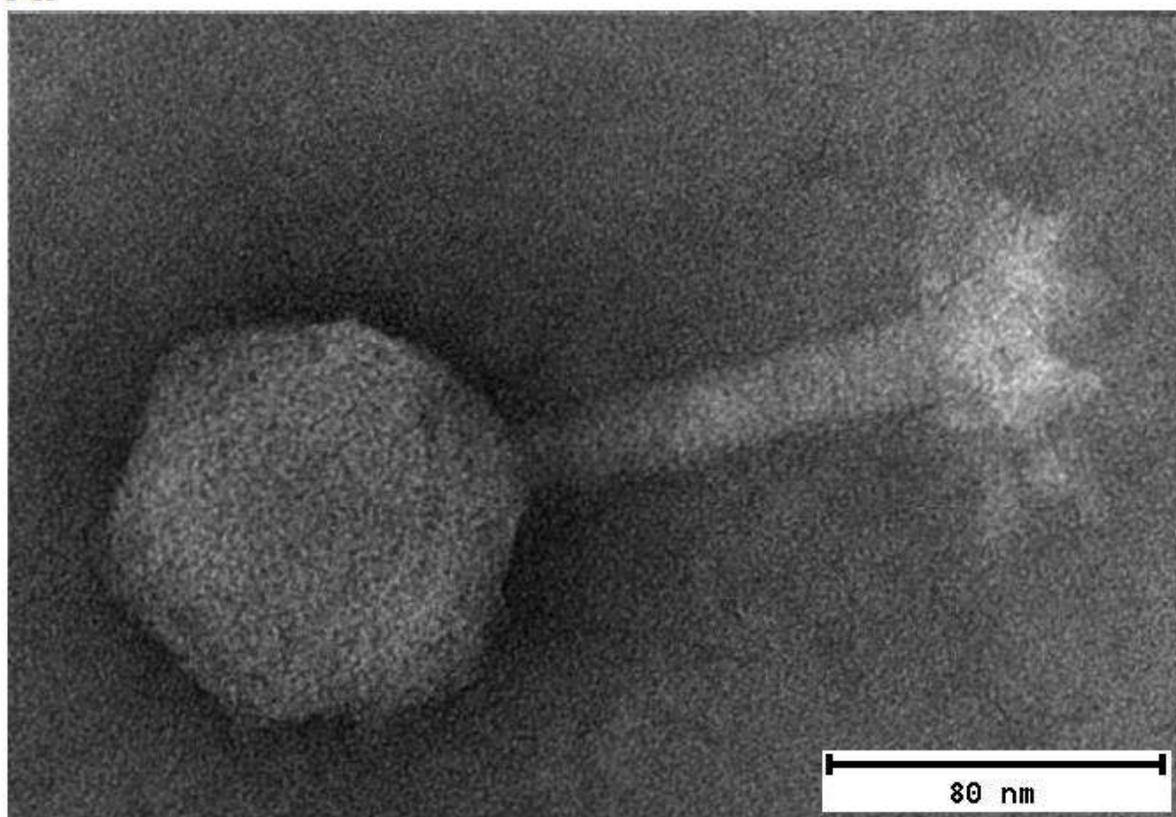
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Figure Legends

Figure 1. Electronic microscopy micrographs of phages. A. vB_AbaM_3054; scale bar represents 80 nm B. vB_AbaM_3090; scale bar represents 100 nm. Morphology of phage particles was studied using a JEOL 100C electron microscope (Jeol, Akishima-Shi, Tokyo, Japan).

A.



B.

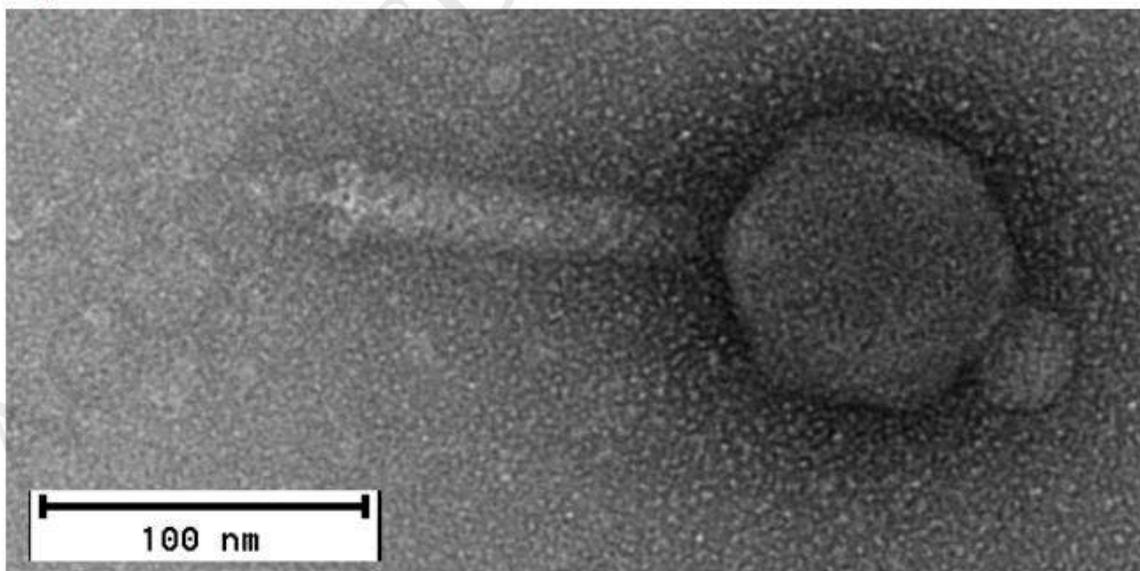
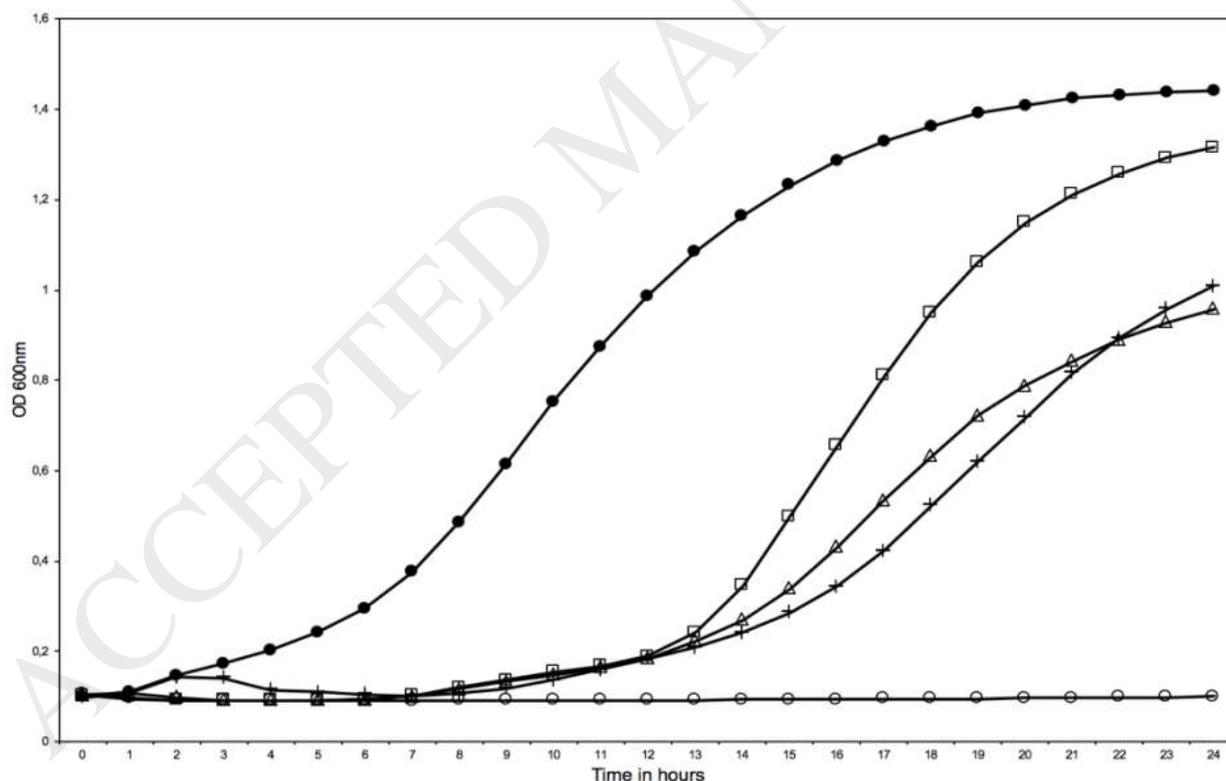


Figure 2. Effect of different treatments on the growth of *A. baumannii* strain FER in a 96-well plate turbidity assay. OD_{600nm} was recorded every 10 min over 24 h. For clarity, only one measurement per hour is indicated. **A. vB_AbaM_3054 at different MOI and imipenem.** Control (NaCl, filled circles); vB_AbaM_3054 (MOI of 100, open squares); vB_AbaM_3054 (MOI of 1, open triangles); vB_AbaM_3054 (MOI of 0.01, crosses); imipenem (167 µg/mL, open circles). **B. vB_AbaM_3090 at different MOI.** Control (NaCl, filled circles); vB_AbaM_3090 (MOI of 100, open squares); vB_AbaM_3090 (MOI of 1, open triangles); vB_AbaM_3090 (MOI of 0.01, crosses). **C. vB_AbaM_3054 and vB_AbaM_3090 at MOI of 100 alone or in combination.** Control (NaCl, filled circles); vB_AbaM_3054 (MOI of 100, open squares); vB_AbaM_3090 (MOI of 100, open triangles); vB_AbaM_3054 + vB_AbaM_3090 (MOI of 50 each, open diamonds). Each dot represents the mean of three independent experiments.



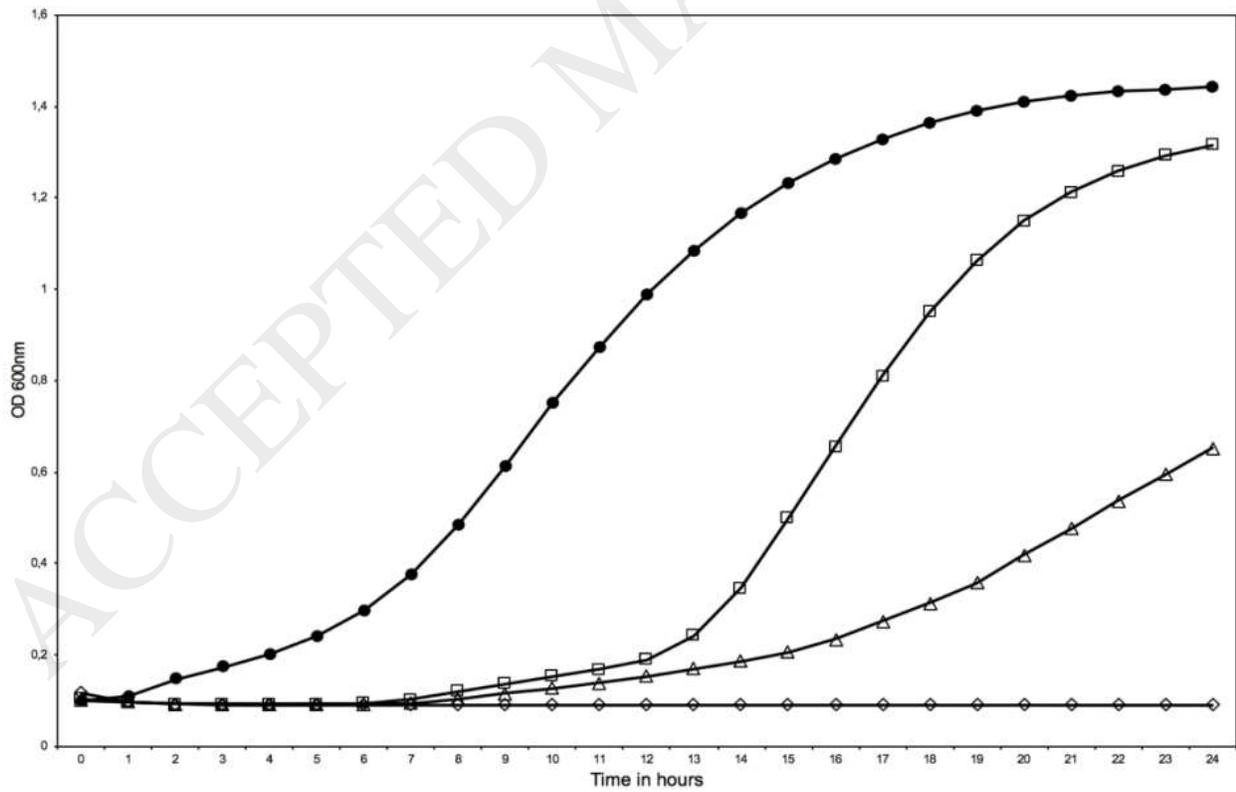
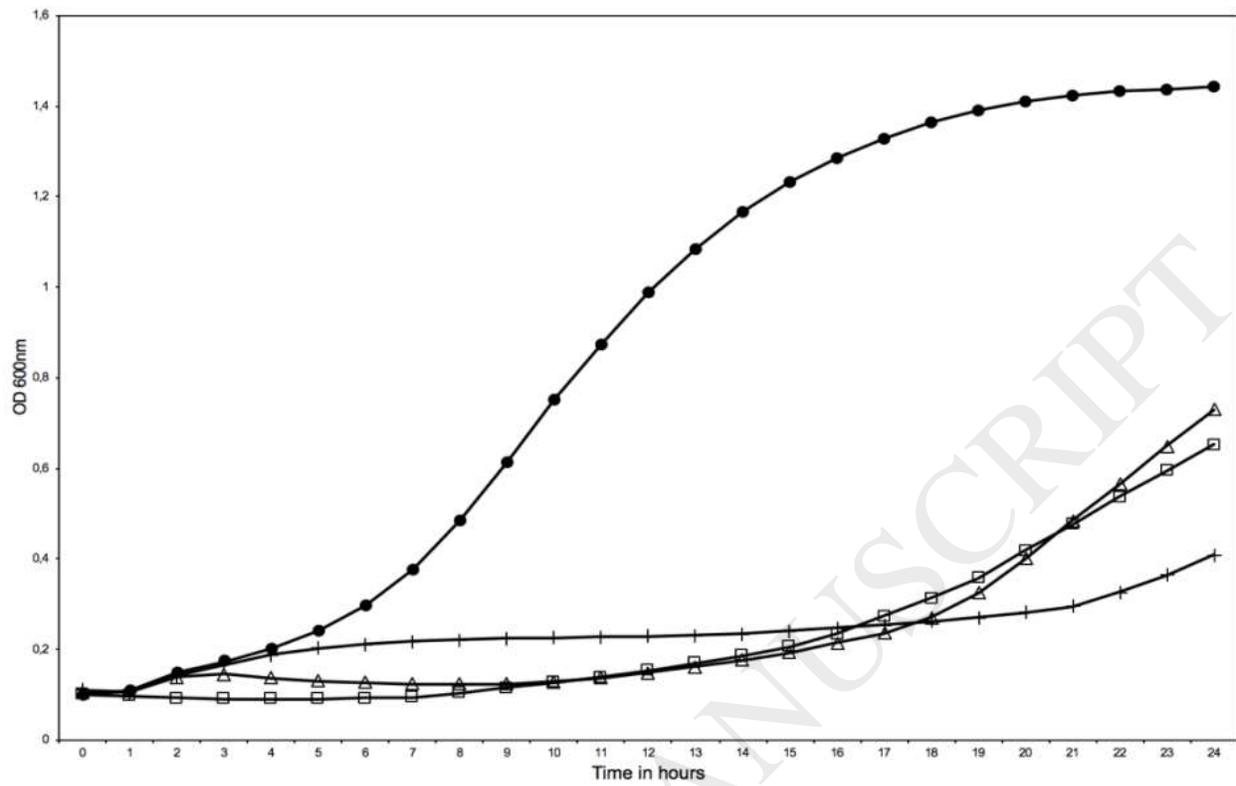


Figure 3. Efficacy of phage therapy in a *Galleria mellonella* model of *A. baumannii* bacteremia. Wax moths (n = 18) were injected in the last right pseudopod with $5 \cdot 10^5$ CFU *A. baumannii* FER. 30 min. later, they received in the left pseudopod either NaCl (control, closed circles), vB_AbaM_3054 (MOI of 100, open triangles), vB_AbaM_3090 (MOI of 100, closed triangles), vB_AbaM_3054 + vB_AbaM_3090 (MOI of 50 each, open squares) or imipenem (5 mg/mL, closed diamonds). Larvae were monitored for survival over a period of 80 h and results were plotted as Kaplan–Meier survival curves. Curves were compared with the log-rank (Mantel–Cox) and Gehan–Breslow–Wilcoxon tests. All treatments groups were significantly different compared to the untreated control group ($p < 0.0001$). There were no statistically significant differences between treatments ($p = 0.3222$).

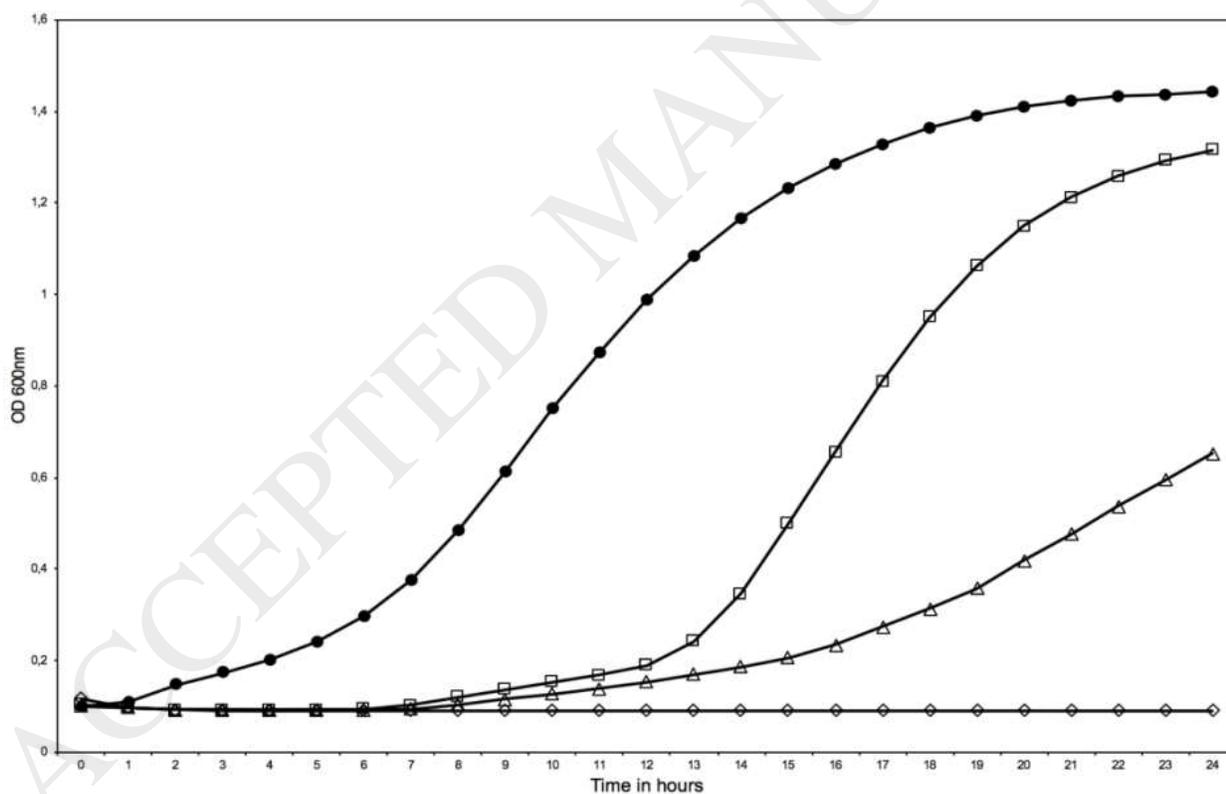


Figure 4. Efficacy of phage therapy in a mouse model of *A. baumannii* bacteremia. CD1 Swiss mice (n = 6) were injected i.p. with ca. $6 \cdot 10^7$ CFU of *A. baumannii* FER. At 2 h post infection, animals received an intraperitoneal injection of either NaCl (control, closed circles), vB_AbaM_3054 (MOI of 100, open triangles), vB_AbaM_3090 (MOI of 100, closed triangles),

vB_AbaM_3054 + vB_AbaM_3090 (MOI of 50 each, open squares) or imipenem (5 mg/mL = 50 mg/kg, closed diamonds). Mice were monitored for survival over a period of seven days and results were plotted as Kaplan–Meier survival curves. Survival curves were compared with the log-rank (Mantel–Cox) and Gehan–Breslow–Wilcoxon tests. All phage treatments groups were significantly different compared to the untreated control group ($p < 0.0001$). There were no statistically significant differences between phage treatments ($p = 0.5913$). Imipenem group was not statistically significantly different to the untreated control group ($p = 0.3173$).

