REVIEW

Specific and Selective Bacteriophages in the Fight against Multidrug-resistant *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii causes serious infections especially in immunocompromised and/or hospitalized patients. Several *A. baumannii* strains are multidrug resistant and infect wounds, bones, and the respiratory tract. Current studies are focused on finding new effective agents against *A. baumannii*. Phage therapy is a promising means to fight this bacterium and many studies on procuring and applying new phages against *A. baumannii* are currently being conducted. As shown in animal models, phages against multidrug-resistant *A. baumannii* may control bacterial infections caused by this pathogen and may be a real hope to solve this dangerous health problem.

Keywords *Acinetobacter baumannii* · Bacteriophages · Phage therapy · Multidrug resistance (MDR)

Acinetobacter baumannii as a Severe Epidemiological Problem

At the beginning of 2019, the World Health Organization published a list of the ten most serious threats to public health (WHO 2019). Antibiotic resistance appeared on this list. *Acinetobacter baumannii* was classified within the most dangerous critical priority group of multidrug-resistant (MDR) bacteria, which may be resistant to third generation cephalosporins and carbapenems (WHO 2018).

The resistance of *A. baumannii* strains was based on three mechanisms: inactivation of antimicrobial enzymes, mutagenesis that changed antimicrobial targets or cellular functions in bacterial cells (efflux pumps and other protein expression), and protection of bacterial targets against

antimicrobial factors (Rice 2006). As an example of the first mechanism, there are genes encoding enzymes called β -lactamases, which hydrolyze the β -lactam bound in carbapenems, cephalosporins, and penicillins (Bonomo and Szabo 2006; Thomson and Bonomo 2005). The second mechanism is related to mutations in the bacterial chromosome affecting bacterial function or bacterial cell targets for antimicrobials. Spontaneous mutagenesis can lead to higher expression of proteins building the efflux pumps and also to affinity reduction for antimicrobial factors (Li *et al.* 2005). The described mechanisms protected *Acinetobacter* cells against quinolone (Bonomo and Szabo 2006) and colistin (Li *et al.* 2005). The third mechanism consists of modification of outer membrane proteins (e.g., porin channel proteins) necessary for transport of antimicrobial agents into bacterial cells (Bou *et al.* 2000; Thomson and Bonomo 2005). The absence of the CarO protein (29 kDa) in the outer membrane of *A. baumannii* led to meropenem and imipenem resistance (Mussi *et al.* 2005). As a result of antibiotics overuse, bacteria are acquiring new and more sophisticated resistance mechanisms, which make them almost impossible to destroy using most available antibiotics.

Infections with MDR bacteria among severely ill patients in intensive care units cause high death risk ranging from 26% to 68% (Kwon *et al.* 2007; Seifert *et al.* 1995; Sunenshine *et al.* 2007). Additionally, mortality

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among patients infected with MDR *A. baumannii* was higher than for infections with MDR *Pseudomonas aeruginosa* (Gkrania-Klotsas and Hershov 2006). Interestingly, MDR strains of *A. baumannii* cause infections of the respiratory tract, bones, bacteremia, or wound infections, which were noted among seriously injured soldiers in Afghanistan and Iraq (CDC 2004; Davis *et al.* 2005; Hawley *et al.* 2007; Hujer *et al.* 2006).

A. baumannii has been classified as an opportunistic bacterium that did not usually infect healthy people (Towner 2009). However, higher mortality has been reported for *A. baumannii* infections than for infections with other gram-negative bacteria (e.g., *Klebsiella pneumoniae*) (Jerassy *et al.* 2006; Robenshtok *et al.* 2006). Multidrug resistance and resistance to unfavorable environmental conditions may lead to frequent nosocomial infections, such as urinary tract and wound infections, pneumonia, bacteremia, or meningitis (Fournier and Richet 2006; Jawad *et al.* 1996). Moreover, high virulence in *A. baumannii* was also associated with biofilm formation, which contributes to difficulties during treatment (Tomaras *et al.* 2003). The pathogen spreads in a medical environment. Infectious strains have been found on laryngoscopes, keyboards, curtains, mops, equipment for patient lifting, and door handles (Wilks *et al.* 2006). Current research showed that MDR *A. baumannii* is also an airborne bacterium present in intensive care units (Jiang *et al.* 2018). Equipment and procedures necessary in patient care (*i.e.*, respiratory equipment, humidifiers, wound care) are serious infectious factors which facilitate the spread of this extremely dangerous pathogen (Aygün *et al.* 2002; Bernards *et al.* 2004; Das *et al.* 2002; De Jong *et al.* 2004; Denton *et al.* 2005; Maragakis *et al.* 2004; Podnos *et al.* 2001; Villegas and Hartstein 2003; Wilks *et al.* 2006; Zanetti *et al.* 2007).

Searching for Phages against *A. baumannii*

Based on the literature (Ghajavand *et al.* 2017) the best source of *A. baumannii* phages is hospital sewage. However, procurement and amplification of these phages entail many difficulties. Therefore, it is extremely important to adjust and optimize these methods for each individual phage. The methods for phage isolation and use are presented in Fig. 1.

Multidrug resistance among *A. baumannii* strains poses serious difficulties for treating infections (Perez *et al.* 2007). However, phage therapy could be a new weapon in the fight against *A. baumannii* infection (Sulakvelidze *et al.* 2001). Bacteriophages have no harmful effects on patients' microbiome and are known to be specific and selective to pathogens. Phage therapy against *A. baumannii* infection

has therefore high potential to be an effective, natural, and safe treatment for patients with serious chronic infections (Clark and March 2006; Międzybrodzki *et al.* 2012; Van Helvoort 1992). Basic information about bacteriophages against *A. baumannii* is presented in Table 1.

Ghajavand *et al.* (2017) examined bacteriophages against *A. baumannii* isolated from intensive care units of Isfahan Medical University hospitals. The study collected 350 samples including urine, catheter, wound, blood, eye swabs, sputum, and cerebrospinal fluid for *A. baumannii* isolation. Examination of multidrug resistance of 43 isolates showed that 100% were resistant to ciprofloxacin; 93% were resistant to meropenem, imipenem, ampicillin-sulbactam, and cefepime; 91% were resistant to trimethoprim-sulfamethoxazole; 86% were resistant to ceftazidime; 84% were resistant to tetracycline; and 54% were resistant to amikacin. Bacteriophages in these studies were obtained from water samples (environmental samples and hospital waste). After incubation of waste samples from hospitals, clear plaques formed on lawns of AB78 and AB39 bacterial strains. Twelve *A. baumannii* isolates were sensitive to IsfAB78 phage and 11 were sensitive to IsfAB39 phage. Morphology of both lytic phages was examined with transmission electron microscopy, which indicated that both phages had six-sided symmetry. They significantly reduced the turbidity of the bacterial culture from OD₆₀₀—2.8 in the control to OD₆₀₀—0.4 in *A. baumannii* cultures treated with IsfAB78 or IsfAB39 phage, which suggested that the examined phages have potential for application in phage therapy. Moreover, studies by Hernandez-Morales *et al.* (2018) resulted in isolation and characterization of Petty phage obtained from sewage. The latent period for this phage was 25 min and burst size was estimated as 240 particles. Among 40 *Acinetobacter* strains tested, 4 were multidrug resistant (resistance to cefazolin, cefotaxime, chloramphenicol, and tetracycline) and susceptible to Petty phage.

Merabishvili *et al.* (2014) described two phages (Acibel004 and Acibel007) selected from wastewater samples. Two *A. baumannii* strains isolated from the nose of a Queen Astrid Military Hospital patient were used for phage amplification. A host range study showed that non-*A. baumannii* strains (*A. nosocomialis* and *A. pittii*) were resistant to both phages. Out of 28 chosen *A. baumannii* strains, 2 were resistant and 15 were sensitive to the phages. Adsorption and/or propagation of phages was possible on 11 strains. Acibel007 propagated on 61% and adsorbed on 71% of 28 *A. baumannii* strains, while Acibel004 propagated on 75% and adsorbed on 89% of strains. Based on efficiency of the plating method, phage Acibel007 had higher lytic activity than Acibel004. Moreover, *A. baumannii* 070517/0072 showed low frequency of mutation during phage application, which suggests low capacity for

Fig. 1 Phages against *Acinetobacter baumannii* and their potential application in medicine

selection of phage-resistant mutants among bacteria. Maximum adsorption for Acibel007 and Acibel004 was 95% in 10 min and 85% in 15 min, respectively. The latent period and burst size for Acibel007 were 21 min and 145 virions per infected bacterium, respectively, and for Acibel004 they were 27 min and 125 virions per host cell, respectively. Bacteria were incubated either with a mixture of two phages or with a single phage. After 24 h of incubation, OD₆₀₀ was 0.13, 0.18, and 0.07 for Acibel004, Acibel007, and simultaneous incubation, respectively, compared to 0.42 in an untreated control. OD₆₀₀ measurements suggested that simultaneous phage application resulted in greater bacterial titer reduction than single phage application.

Turner *et al.* (2017) characterized another phage against *A. baumannii*, the bacteriophage Loki (vB_AbaS_Loki), isolated from sludge. The latent period for Loki phage was 40 min and burst size was 43 PFU per infective center. Mutation in *A. baumannii* ATCC 17978 gene *lpxA*, encoding a protein required for LPS synthesis, prevented phage adsorption. The authors suggested that an LPS component could be used by Loki as a receptor on the bacterial host. Loki formed turbid plaques (0.5 mm in diameter) on *A. baumannii* ATCC 17978 and Ca²⁺ was necessary for plaque formation. Thirty-six out of 38 examined *A. baumannii* strains were sensitive to Loki phage. Genome analysis showed that Loki has genes *gp50* (a putative class I holin) with two transmembrane domains and *gp51* containing an N-acetylmuraminidase and a domain that binds peptidoglycan at the C-terminus (Briers *et al.* 2007).

Hua *et al.* (2018) looked for phages against MDR *A. baumannii* clinical isolates. They found that 48 isolated

strains were resistant to ciprofloxacin, cefepime, ceftazidime, and piperacillin-tazobactam. The study obtained 30 bacteriophages but only four of them were described: phage SH-Ab 15599, SH-Ab 15708, SH-Ab 15497, and SH-Ab 15519. The phage cocktail containing all these phages was effective against 88% of *A. baumannii* isolates but no synergic effect was described (*i.e.*, strains resistant to single phage application were also resistant to the phage cocktail). Phages SH-Ab 15599, SH-Ab 15708, SH-Ab 15497, and SH-Ab 15519 were effective against 27%, 29%, 29%, and 17% of *A. baumannii* isolates, respectively. For phage SH-Ab 15519, an enzyme was suggested as cause of bacterial exopolysaccharide degradation. This phage demonstrated high absorption (90%) within 10 min. Subsequently, the phage was shown to be stable at pH 5 to 12 and temperatures from 4 to 50 °C.

Kusradze *et al.* (2016) described another phage against *A. baumannii*, phage vB-GEC_Ab-M-G7 (*phi* G7), which was classified in the *Myoviridae* family (Matsuzaki *et al.* 2005). The phage proved to be active against 65% of the 200 *A. baumannii* strains tested (Kusradze *et al.* 2016). Phage activity was determined at different temperatures and it was observed that incubation at 37 °C had no effect on phage activity. After 24 h incubation at 50 °C, 90% of phage particles were still active but total inactivation was obtained after 24 h incubation at 70 °C. Moreover, phage *phi* G7 remained stable when incubated for 24 h in the presence of chloroform and for 5 h at pH from 5 to 11. Phage titer reduction to 10³ was caused by 24 h incubation at pH 3.

Cha *et al.* (2018) examined the sensitivity of 14 MDR *A. baumannii* strains to nine bacteriophages: PBAB05,

Table 1 Bacteriophages against *A. baumannii*.

Phage symbol	Family	Morphology	Source of isolation	Number of tested strains	Type of life cycle	Animal model application	References
IstAB78	<i>Myoviridae</i>	Six-sided symmetry, 100 nm long	Water sample	43 MDR strains (12 of those strains were sensitive to phage)	Lytic	N.A.	Ghajavand <i>et al.</i> (2017)
IstAB39	<i>Podoviridae</i>	Six-sided symmetry, short tail and 50 nm head	Water sample	43 MDR strains (11 of those strains were sensitive to phage)	Lytic	N.A.	
Petty phage	<i>Podoviridae</i>	Short 15 nm long tail and 60 nm head	Sewage	40 strains (25 of those strains were MDR; 4 strains were sensitive to phage)	N.A.	N.A.	Hernandez-Morales <i>et al.</i> (2018)
Acibel004	<i>Myoviridae</i>	105 nm long contractile tail and 70 nm icosahedral head and formed 1–2 mm plaques	Wastewater sample	34 MDR strains (28 of those strains were sensitive to phage)	Lytic	N.A.	Merabishvili <i>et al.</i> (2014)
Acibel007	<i>Podoviridae</i>	10 nm long noncontractile tail, 60 nm head and forms 3–5 mm plaques	Wastewater sample	34 MDR strains (28 of those strains were sensitive to phage)	Lytic	N.A.	
vB_AbaS_Loki	<i>Siphoviridae</i>	Isometric 57 nm capsid and non-contractile 176 nm long tail (and 10 nm diameter); short spikes at the tail terminus	Sludge	34 strains (2 of those strains were sensitive to phage)	Lytic	N.A.	Turner <i>et al.</i> (2017)
SH-Ab 15599	<i>Myoviridae</i>	Head with tail (both 88 nm long), formed round, clear plaques (2–3 mm diameter) with haloes	Sewage	48 carbapenem resistant strains (13 of those strains were sensitive to phage)	Lytic	N.A.	Hua <i>et al.</i> (2018)
SH-Ab 15708	<i>Myoviridae</i>	Head (88 nm long diameter) and tail (63 nm long) formed round, clear plaques (2–3 mm diameter) with haloes	Sewage	48 carbapenem resistant strains (14 of those strains were sensitive to phage)	Lytic	N.A.	
SH-Ab 15497	<i>Siphoviridae</i>	Tail (125 nm long and 4 nm wide) and head (55 nm diameter) formed small plaques (0.5 mm diameter)	Sewage	48 carbapenem resistant strains (14 of those strains were sensitive to phage)	Lytic	N.A.	
SH-Ab 15519	<i>Podoviridae</i>	Short, non-contractile, straight tail (18 nm long) and polyhedral, symmetrical head (55 nm diameter) formed round, clear plaques (8–9 mm diameter) with haloes	Sewage	48 carbapenem resistant strains (8 of those strains were sensitive to phage)	Lytic	Mouse model- lung infection; intranasally administration of phage; the survival rate 90%	

Table 1 continued.

Phage symbol	Family	Morphology	Source of isolation	Number of tested strains	Type of life cycle	Animal model application	References
νB- <i>GEC_Ab-M-G7</i> ^{was} (<i>phi G7</i>)	<i>Myoviridae</i>	120 nm contractile tail and 100 nm diameter icosahedral head	Sewage water	200 strains (136 of those strains were sensitive to phage)	Lytic	Rats' wound model; phage added on the wound; the survival rate 100%	Kusradze et al. (2016)
PBAB08	<i>Myoviridae</i>	Head 180 nm in diameter and 360 nm long tail	Bacteriophage Bank of Korea	14 MDR strains (5 of those strains were sensitive to phage)	N.A.	Mice model- lung infection; intranasal phage cocktail (PBAB08, PBAB25, PBAB68, PBAB80, PBAB93) injection; the survival rate 35%	Cha et al. (2018)
PBAB25	<i>Myoviridae</i>	Head of 80 nm in diameter and 90 nm long tail	Bacteriophage Bank of Korea	14 MDR strains (1 of those strains were sensitive to phage)	N.A.	Mice model- lung infection; intranasal phage cocktail (PBAB08, PBAB25, PBAB68, PBAB80, PBAB93) injection; the survival rate 35%	Wu et al. (2019)
PD-6A3	<i>Podoviridae</i>	Short tail (9 nm in length), isometric head (50 nm in diameter); the phage created significant 2–3 mm diameter halos	Sewage	552 MDR strains (179 of those strains were sensitive to phage)	Lytic	Sepsis mouse model; intraperitoneal administration; the survival rate of endolysin therapy group, endolysin + phage therapy group, phage therapy group and phage cocktail (14 phages) therapy group were 70, 70, 60, and 50%, respectively	Jasim et al. (2018)
AB3P1	N.A.	0.8–1.5 mm oval plaques	Different regions in Baghdad city including sewage, farm soil, feces of sheep, chicken litter, and swab from surgical lounge of several hospitals in Baghdad	23: 11 extensive- and 12 pan-drug resistant strains (18 of those strains were sensitive to phage)	Lytic	Mice model; intraperitoneally administration of AB3 phages; the survival rate 100%	Jasim et al. (2018)
AB3P2	N.A.	0.5 mm oval plaques	Different regions in Baghdad city including sewage, farm soil, feces of sheep, chicken litter, and swab from surgical lounge of several hospitals in Baghdad	23: 11 extensive- and 12 pan-drug resistant strains (18 of those strains were sensitive to phage)	Lytic	Mice model; intraperitoneally administration of AB3 phages; the survival rate 100%	Jasim et al. (2018)

Table 1 continued.

Phage symbol	Family	Morphology	Source of isolation	Number of tested strains	Type of life cycle	Animal model application	References
AB3P3	N.A.	2.5–4.0 mm round plaques	Different regions in Baghdad city including sewage, farm soil, feces of sheep, chicken litter, and swab from surgical lounge of several hospitals in Baghdad	23: 11 extensive- and 12 drug resistant strains (18 of those strains were sensitive to phage)	Lytic	Mice model; intraperitoneally administration of AB3 phages; the survival rate 100%	
AB3P4	N.A.	35 mm oval plaque	Different regions in Baghdad city including sewage, farm soil, feces of sheep, chicken litter, and swab from surgical lounge of several hospitals in Baghdad	23: 11 extensive- and 12 drug resistant strains (18 of those strains were sensitive to phage)	Lytic	Mice model; intraperitoneally administration of AB3 phages; the survival rate 100%	

PBAB06, PBAB07, PBAB08, PBAB25, PBAB68, PBAB80, PBAB87, and PBAB93. Seven of the tested bacterial strains were sensitive to four or five phages. *A. baumannii* 28 was sensitive to five out of nine phages. These bacteriophages were used to prepare a phage cocktail, which was used in *in vivo* studies. Burst size for PBAB25 and PBAB08 was 630 and 215, respectively, and both phages were able to cause bacterial cell lysis 25 min after infection. PBAB08 and PBAB25 showed stability at pH 5–10 and temperatures from 4 to 55 °C for 1 h. Moreover, the authors sequenced whole genomes of phages PBAB08 and PBAB25 and they found that the genome of these phages was linear, with dsDNA of 42312 bp (PBAB08) and 40260 bp (PBAB25). Sequenced genomes were compared with reported genomes of phages against *Acinetobacter*. The results showed that phage PBAB08 had 57% sequence coverage and 99% similarity to AB1 phage (Yang *et al.* 2010), whereas phage PBAB25 had 78% sequence coverage and 97% similarity to IME_AB3 phage (Zhang *et al.* 2015).

Wu *et al.* (2019) presented the characterization of 14 lytic bacteriophages, which were tested on 552 *A. baumannii* strains. The phage cocktail combining the mentioned phages lysed 54% of 552 bacterial isolates. One of the lytic phages (phage PD-6A3) had a wider lytic spectrum (32% of 552 bacterial isolates) than the other 13 bacteriophages. PD-6A3 bacteriophage was stable at temperatures between 4 and 50 °C and at pH from 5 to 10. The burst size for this phage was 129 PFU per infected bacterial cell.

Liu *et al.* (2019) described another phage against *A. baumannii*. Phage IME200 formed 2 mm diameter clear plaques with halos. The halo was associated with depolymerase activity. Phage IME200 was able to infect 10 out of 41 *A. baumannii* isolates and it encoded an enzyme that might be useful in the treatment of biofilm formed by *A. baumannii* strains. This biofilm is resistant to disinfectants, so the use of phages with depolymerizing genes in their genomes creates a chance for treatment of infections caused by biofilm-forming resistant *A. baumannii* strains.

In another study, Jasim *et al.* (2018) isolated 23 *A. baumannii* strains from patients with septicemia, urinary tract infection, meningitis, wound infection, or pneumonia. Twelve out of 23 isolates were pan-drug resistant and 11 of them were extensive-drug resistant. Pan-drug-resistant clinical isolates are defined as strains causing difficulties in treatment and resistant to all antimicrobial drugs (Göttig *et al.* 2014). Analyses of bacterial sensitivity to bacteriophages indicated that 111 out of 136 *A. baumannii*-specific bacteriophages had high lytic properties and formed clear plaques with diameter greater than 3 mm (Jasim *et al.* 2018). For 10 randomly selected bacteriophages, the burst time ranged from 30 to 45 min and the minimum and

maximum burst size was 130 and 245, respectively. Moreover, a phage cocktail (64 bacteriophages specific against *A. baumannii* isolates) was examined on 23 isolates (AB1-AB23). The results showed that 21 bacterial isolates (except AB2 and AB8) were infected by more than one bacteriophage, and AB3 was infected by six bacteriophages.

Wang *et al.* (2018) studied the use of phage ϕ m18p against *A. baumannii* clinical isolate KM18. Murine RAW 264.7 macrophages were infected with KM18 and treated with the phage ϕ m18p. After phage application [multiplicity of infection (MOI) 4, 0.4, and 0.04], the cell number was around two times higher than among cells infected with bacteria without treatment. *A. baumannii* titer in infected cells without treatment was around 9 log CFU/mL, but in cells treated with phage at MOI of 4, 0.4, and 0.04 it was around 4, 6, and 7 log CFU/mL, respectively.

Effectiveness of Phage Therapy Assessed in Animal Models of *A. baumannii* Infection

Among the four bacteriophages characterized by Hua *et al.* (2018), SH-Ab 15519 was chosen for treatment in a mouse model of lung infection. *A. baumannii* was administered by the intratracheal route to neutropenic mice. The level of survival among mice infected with 10^6 CFU of *A. baumannii* was 91%. The survival level among mice infected with 10^7 , 10^8 , and 10^9 CFU of bacteria was 36%, 18%, and 18% respectively. Subsequently, mice were also infected with 10^8 CFU of *A. baumannii* and phage SH-Ab 15519 was administered intranasally at 0.1, 1, and 10 MOI. Different phage doses significantly increased the survival level among mice to the same value (90%) compared to the non-treated control (10%).

The potential of *phi* G7 phage application was examined in a rat wound model (Kusradze *et al.* 2016). In the control group, with an artificial wound and without inoculation of *A. baumannii*, no mortality was detected. No toxic effect after phage application was noted and all wounded rats treated with phages survived. Samples taken from rats using a sterile swab during the experiment showed no contamination with *A. baumannii*. Furthermore, samples from wounds taken using sterile swabs, which were added to 4.5 mL of BHI broth, showed a phage titer not more than $\sim 2 \times 10^1$ PFU/mL of BHI broth. Two groups of rats were infected with bacteria in a titer of 5×10^8 CFU/mL, the first with *A. baumannii* T-10 and the second with *A. baumannii* G7. After 2 days, inflammation and pus formation were noted. Infection with *A. baumannii* G7 was more complicated than *A. baumannii* T-10 infection. In the untreated group infected with bA. *baumannii* G7, 70% of rats survived 5 days after inoculation. For animals infected

with *A. baumannii* G7 and treated with 5×10^7 PFU/mL phage *phi* G7 12 h after infection, no mortality was reported and there were no purulent lesion after 6 days of the experiment. Furthermore, bacterial titer was reduced to 3×10^2 CFU/mL. In the group infected with the *A. baumannii* T-10, phage therapy led to full recovery of animals before 5 and 6 days of experiment (bacterial titer decreased to 9×10^2 CFU/mL).

A phage cocktail (consisting of five phages against *A. baumannii* 28) was used to treat mice infected intranasally with 1×10^8 CFU of *A. baumannii* 28 (Cha *et al.* 2018). Phages were also administered intranasally (1×10^9 PFU) every day of the experiment (7 days). At days 0 and 1, the phage cocktail was administered 4 h after bacterial infection. In the control group (*i.e.*, mice infected with bacteria without phage therapy), the survival rate was 15% compared to mice treated with the phage cocktail where survival rate was 35%, 7 days after *A. baumannii* infection. Moreover, the bacterial titer (*i.e.*, bacteria resistant to ampicillin and kanamycin) in mice lungs was reduced more than 100-fold, at 3 and 4 days after infection. Intranasal and oral phage administration had no effect on IgE level in mice serum; only intraperitoneal injection caused a 20% increase of IgE, which may suggest the potential route of phage administration. Furthermore, Soothill (1992) described *in vivo* studies concerning BS46 phage action against *A. baumannii* AC54. In the first study, mice were injected into the peritoneal cavity with 0.25 mL of 8 LD50 (LD50 for *A. baumannii* AC54 = 1.9×10^7 CFU). Five mice couples were treated with either 8.3×10^6 , 1.7×10^6 , 3.3×10^5 , 6.6×10^4 , or 1.3×10^4 PFU of phage BS46, and one last couple had no treatment with phage BS46. All mice in the treatment group survived, whereas all mice died in the non-treatment group. In the second study mice were injected with 0.25 mL of 3 LD50—five groups with one mouse per group. Each mouse was treated with either 1200, 120, 12, or 1 PFU of phage BS46, and the last mouse had no treatment with phage BS46. The first three mice survived but the last two died. Moreover, the author described a third study where mice were injected with 0.25 mL of 3 LD50—five groups with five mice per group. Each group was treated with 108, 36, 12, or 4 PFU of phage BS46, and the last group had no treatment with phage BS46. Mice survived only in the first group (four out of five treated mice), while all mice died in the other groups. Additionally, there was no toxicity of the phage BS46 for mice in the control group (*i.e.*, treated only with phage). No differences in health status between mice with phage therapy and without phage administration were observed.

The phage PD-6A3, a phage cocktail, and Ply6A3 endolysin were tested in intraperitoneally infected mice (sepsis mouse model) with 1×10^9 CFU/mL of *A.*

baumannii 32 (Wu *et al.* 2019). The survival rate among mice treated with Ply6A3 endolysin (intraperitoneal administration of 1 mL Ply6A3 at 2 mg/mL), Ply6A3 endolysin with PD-6A3 phage (intraperitoneal administration of 1 mL Ply6A3 at 2 mg/mL and 1 mL PD-6A3 at 10^9 PFU/mL), PD6A3 phage (intraperitoneal administration of 1 mL PD-6A3 at 10^9 PFU/mL), and phage cocktail (intraperitoneal administration with 1 mL of phage cocktail at 10^9 PFU/mL) was 70%, 70%, 60%, and 50%, respectively, on the last (7th) day of the experiment. Mice in the control group (infected with *A. baumannii* 32) died on the third day of the experiment.

Jasim *et al.* (2018) described *in vivo* studies where mice were injected intraperitoneally with AB3 isolate (0.3 mL at 10^6 CFU/mL) and treated, also intraperitoneally, with phage specific to the AB3 strain (0.3 mL at 10^8 PFU/mL administered 2 h after phage injection). All mice in this group survived more than 6 weeks. Contrastingly, all mice died 4 days after infection in the group without phage therapy. Moreover, in the group treated with phage but without bacterial infection mice also survived. *In vivo* studies of BALB/c mice showed that KM18 intraperitoneal injection at $2\text{--}2.5 \times 10^8$ CFU resulted in mice death after 24 h. Infection with KM18 at $2\text{--}3 \times 10^7$ CFU resulted in a survival rate of 50% after 14 days of the experiment, but after infection at $2\text{--}3 \times 10^6$ CFU all mice survived 14 days of the experiment (Wang *et al.* 2018). Phage therapy with ϕ m18p at different MOI (*i.e.*, 10, 1, and 0.1) protected mice infected with $2\text{--}3 \times 10^8$ CFU of KM18 (100% of mice survived 14 days of experiment). Moreover, levels of cytokines in mice infected with *A. baumannii* were high (tumor necrosis factor α [TNF- α] = 3000 pg/mL; interleukin 6 [IL-6] = 35000 pg/mL). However, mice treated with phage had low levels of TNF- α and IL-6 (250 pg/mL and 1000 pg/mL, respectively), even at the lowest MOI. This showed that the phage was able to increase the survival rate among mice infected with *A. baumannii* and also reduced the inflammatory response of infected mice.

All of these animal models have demonstrated that phage therapy can efficiently eliminate *Acinetobacter* infection, which creates an opportunity for future clinical application in humans.

Endolysins against *A. baumannii*

The studies on phage PD-6A3 showed that it encoded Ply6A3 endolysin, which degraded peptidoglycan (forming a transparent ring) compared to a negative control (Wu *et al.* 2019). The phage ($10^6\text{--}10^{10}$ PFU/mL) and endolysin (0.25–2.0 mg/mL) were incubated with HEK293T (human renal epithelial cells) and THP-1 (human mononuclear

macrophage) cells for 8 and 24 h to study potential cytotoxicity of PD-6A3 and Ply6A3. The results showed that the level of viability for both types of cells with phage and endolysin was around 100% after both 8 and 24 h of incubation. This showed PD-6A3 and Ply6A3 safety of use. Moreover, the endolysin was able to lyse such bacterial strains as *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli*, and *Klebsiella pneumoniae*.

Furthermore, Liu *et al.* (2019) reported that 10 out of 41 *A. baumannii* isolates were sensitive to depolymerase Dpo48. Activity of Dpo48 was examined under different pH conditions and the optimal pH was determined to be 5.0, although Dpo48 was active at pH from 5.0 to 9.0. The optimal temperature range for this depolymerase was 20 °C–70 °C. Additionally, the authors showed that overnight incubation of *A. baumannii* AB1610 (10^9 CFU/mL) with Dpo48 (10 μ g/mL) and 1 h incubation with 50% human serum led to a five-log reduction of bacterial titer compared to the control with inactive serum. This showed the important role of complement combined with endolysin in bacterial killing.

Clinical Application of Bacteriophages against *A. baumannii*

Using a purified phage cocktail, Schooley *et al.* (2017) combined local and intravenous phage therapy application for the first time on a 68-year-old diabetic patient with necrotizing pancreatitis and pancreatic pseudocyst complicated by MDR *A. baumannii* infection. Three pathogenic *A. baumannii* strains (TP1, TP2, and TP3) were isolated from the patient's pancreatic drainage. Among 98 lytic bacteriophages against TP1, TP2, and TP3, nine phages were selected to obtain three phage cocktails. Φ PC cocktail was administered for 18 weeks from day 109 of the illness to drain the gallbladder, pseudocyst cavity, and third intra-abdominal cavity through percutaneous catheters. Φ PC contained AC4, C1P12, C2P21, and C2P24 *Myoviridae* phages against the TP1 strain. Φ IV cocktail was administered intravenously at 5×10^9 PFU for 16 weeks from day 111 of the illness. Φ IV contained AB-Navy1, AB-Navy4, AB-Navy71, and AB-Navy97 *Myoviridae* phages against the TP1 strain. Φ IVB cocktail was administered intravenously at 5×10^9 PFU for 2 weeks from day 221 of the illness. Φ IV contained AB-Navy71 (*Myoviridae*) phage against the TP1 strain and AbTP3 Φ 1 (*Podoviridae*) against TP1, TP2, and TP3 strains. Φ PC, Φ IV, and Φ IVB contained 2.4×10^3 , 5.89×10^3 , and 1.64×10^3 EU/mL endotoxin, respectively. Phage therapy was supplemented with minocycline 4 days after the initial Φ PC treatment. Two days after Φ IV administration the patient's condition had improved and the patient awoke

from a coma. Phage therapy was continued for the next 8 weeks and the patient left hospital on day 245. The above results indicate high potential for phage therapy to treat patients with a serious condition in whom MDR bacterial strains were detected. Furthermore, the phages can also restore the sensitivity of bacteria to antibiotics.

Ho et al. (2016) examined phage aerosol activity on *A. baumannii* strains in a hospital in Taiwan. According to this study, eight active phages were selected (Φ AB1, Φ AB2, Φ AB6, Φ AB7, Φ 4C08, Φ 8C07, Φ AB11, and Φ 5C05), which were used sequentially 52 times. During the first period of the experiment (1.01.2012–28.02.2013), 191 new carbapenem-resistant *A. baumannii* strains were identified in intensive care units, and during the second period (1.03.2013–31.12.2013) the number of identified bacterial strains was 73. Cleaning with phage aerosol decreased the rate of new carbapenem-resistant *A. baumannii* from 8.57 to 5.11. Therefore, the use of phage aerosols may be of use for decontamination of hospital surfaces in the future.

Conclusions

Our experience with phage therapy is encouraging; good clinical results may be observed in many cases, while its safety appears to be unquestionable (Górski et al. 2018; Międzybrodzki et al. 2012). Furthermore, the first double-blind, controlled, randomized clinical trial of phage therapy has recently confirmed phage therapy's safety and suggested that phage preparations even with very low phage titers may reduce the burden of bacteria in treated patients (Jault et al. 2018). Results of experimental phage therapy of *A. baumannii* infection in animals (including humans) are scarce, but very encouraging. Evidently, clinical trials are urgently needed to confirm the value of phage therapy against this pathogen in accordance with evidence-based medicine.

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Compliance with Ethics Standards

Conflict of interest Andrzej Górski, is co-inventor of patents owned by the Institute and covering phage preparations. Other authors declare that they have no conflict of interest.

Animal and Human Rights Statement This article does not contain any studies with human or animal subjects performed by any of the authors.

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