Phage Therapy on Human and Poultry Infection

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Acinetobacter baumannii is a multidrug-resistant and invasive pathogen associated with the etiopathology of both an increasing number of nosocomial infections and is of relevance to poultry production systems. Phage therapy has gained particular importance for the treatment of bacterial infections. Phage therapy represents a potential treatment solution for multidrug-resistant *Acinetobacter baumannii.*

multidrug-resistant Acinetobacter baumannii | phage therapy | one health | Poultry | antimicrobial resistance

1. Single Phage Therapy

Therapies based on a single virus type, also known as monophage therapies, have been extensively applied as *A. baumannii* treatments. Jeonet et al. (2012) found that the phage YMC 13/03/R2096 ABABBP or the molar φ-R2096 exhibited high lytic activity against *A. baumannii* growth in a dose-dependent manner [1]. In another study, intranasally administered phage SH-AB15519, originally isolated from hospital wastewater, was found to be effective in treating pneumonia led by carbapenem-resistant *A. baumannii* infection in mice [2]. Interestingly, phage SH-AB15519 has been demonstrated to be lacking genes connected to further virulence or AMR [3], possibly as a symptom of its low integration rate, which might endorse the use of this phage as a possible antibiotic alternative. PD-6A3 is a novel *A. baumannii* phage which also inhibits *Escherichia coli* and Methicillin-resistant bacteria [4]. Furthermore, Phage Abp9 effectively treated the biofilm produced by *A. baumannii* strain ABZY9 in vitro and contributed to positive treatment outputs in a murine model of *A. baumannii* infection [5]. Phage φ KM18P was used in XDR *A. baumannii* bacteraemia models in BALB/C and C57BL/6 mice, where it improved the survival rate of animals and reduced the number of bacteria in the blood, concurring with decreased levels of TNF- α and interleukin-6 [6]. The bacteriophage vB_AbaP_AGC01, isolated from a fish pond sample collected in Stargard (Poland), has been shown to have high specificity to *A. baumannii* and to generate high-yield viral offspring (317 ± 20 plaque-forming units per cell) [7]. The phage vB_AbaP_AGC01, either alone or in combination with antibiotics (gentamicin, ciprofloxacin and meropenem), significantly reduced *A. baumannii* cell counts in a human heat-inactivated plasma model [7]. In parallel, the phage vB_AbaM_PhT2 prevented *A. baumannii*-induced cell damage in human brain and bladder cell lines by significantly reducing bacterial cytotoxicity and the dose of colistin needed [8]. Therefore, these findings suggest that phages in general, and perhaps phage vB_AbaM_PhT2 in particular, could be applied as antibacterial agents in a hospital environment. The bacteriophage STP4-A, screened by Li et

al., has a strong inhibitory effect on both single and multiple salmonella strains and is a safe antibacterial agent with a wide host range which can be used in the poultry industry.

2. Cocktail Therapy

Phage cocktails typically consist of multiple phages combined, with each of them having unique host specificity due to selective affinity towards a specific bacterial receptor, conferring a broad therapeutic lysis spectrum [9]. On the other hand, the development of phage resistance, especially to lytic viruses, should be carefully monitored, and cocktails appear to be a valid approach to limit such occurrences. It has been shown, for example, that a designed cocktail of the phages vB_AbaS_D0, isolated from hospital-sewage samples in Dalian (China), and vB_AbaP_D2 decreased the mutation frequency of *A. baumannii* whilst also decreasing the percentage of phage-resistance in a murine bacteraemia model [10]. Wu et al. reported the the administration of a phage cocktail (φAb121 and φAb124) to four patients in a COVID-19 intensive care unit in China was able to treat carbapenem-resistant *A. baumannii* infection, otherwise showing the insurgence of phage-resistant *A. baumannii* strains when only one phage was administered [11]. The application of a cocktail of bacteriophages has also been demonstrated to be an effective substitute to antibiotic growth promoter replacement in broiler diets [12], which would further assist in the reduction of the development of anti-microbial resistance arising from poultry production.

Similarly, the emergence of anti-phage mutants can be suppressed by ensuring a high titre throughout cocktail treatment. Beyond phage-resistance, another factor to consider is that treatment with high-populated phage cocktails may lead to complex pharmacological and immune responses, which may hinder the implementation of clinical trials [13], hence the recommendation of the use of a less complex cocktail consisting of up to 2–10 phages as the first choice [14]. As observed in other fields, the misuse of antibiotics associated with livestock, including in poultry production, has led to the selection and spread of multi-drug resistant organisms (MDROs), including *A. baumannii* [15]. The zoonosis risk associated with these MDROs is not only clinically relevant to the development of a specific symptomatology, but it could also contribute to the spread of AMR to humans thanks to mechanisms such as, e.g., horizontal gene transfer. Although the use of phage therapy to control *A. baumannii* infection in poultry has not been reported, many studies have been carried out on other pathogens in farm animals. Indeed, Campylobacter jejuni abundance in broilers was decreased by oral treatment with a Campylobacter-specific- phage cocktail, without further affecting microbiota species [16], providing a working example for the further future application of similar strategies to modulate *A. baumannii* overgrowth in poultry and other livestock.

3. Phage–Antibiotic Synergy

Phage–antibiotic synergy (PAS) refers to the usage of antibiotics at sublethal doses in combination with phage administration, with the aim of increasing the release of phage-progeny from bacterial cells [17]. PAS strategies have a number of advantages, such as enhanced bacterial inhibition, the reduced development of phages and the penetration of biofilms [18]. However, care should be taken when considering a combined therapy due to their unavoidable increased risk towards AMR insurgence. Low antibiotic doses used in such combinations could indeed

facilitate the selection of resistant species. Moreover, the impact of these antibiotics on the rest of the microbiota symbionts, beyond the primary target, ought to be taken into consideration [19].

Importantly, the final PAS effect is affected by not only the qualitative distribution of antibiotics in the mix, but also by their relative concentrations. Ma et al. [20]. optimized the multiplicity of infection (MOI, i.e., optimal phage/target ratio) of phages in combination with eight different antibiotics applied to the control of *A. baumannii*, demonstrating that a reduction in the rifampicin concentration led to a decreased PAS effect, which was otherwise increased by a decrease in both meropenem and minocycline concentrations. On the other hand, the effectiveness of PAS, as a combined approach, has been shown in several studies. Indeed, Grygorcewicz et al. observed approximately a 4-log reduction of *A. baumannii* when using vB_AbaP_AGC01 phage in combination to ciprofloxacin and meropenem, in a heat-inactivated plasma blood model [7].

4. Phage-Encoded Enzymes for the Treatment of A. baumannii

4.1. Endolysins

Endolysins are phage-produced hydrolases that lyse bacterial cell walls, allowing the further release of progeny phages at the end of the replication cycle [21]. These enzymes are very effective towards peptidoglycan layers, leading to a sudden drop in osmotic pressure and therefore lysis [22]. According to their action on the main bonds in the peptidoglycan layer, endolysins are divided into five categories: (I) N-acetyl-β-D-intracellular amidase, (II) N-acetyl-β-D-glucosaminidase, (III) transglycosidase; (IV) N-acetyl-leucoyl-l-alanine amidase and (V) L-alaninoyl-D-glutamate endopeptidase [23]. The main advantage of endolysin therapy over traditional broad-spectrum antibiotics is endolysins' high specificity towards bacterial species or subspecies without interacting with the surrounding microbial cells [24]. Additionally, further advantages associated with endolysins are connected to reduced resistance, to their synergistic activity with different antibacterial agents and to their ability to play an effective role on biofilm and the mucosal surface [25].

4.2. Depolymerases

During biofilm formation, bacterial cells are usually surrounded by extracellular polymers (EPSs), which can also act as barriers for phage penetration [26]. *A. baumannii* EPSs increases the resistance of the bacterium to antimicrobial agents due to diffusion limitation and can lead to severe persistent infections that are particularly difficult to treat, with them also providing resistance to phages [27]. Depolymerases are phage-derived enzymes that facilitates the early stages of phage infection by degrading extracellular bacterial protein [28]. The depolymerase responsible for degrading EPSs or O-polysaccharides can be found either as a virion component or it can be secreted in a soluble form during bacterial cell lysis [29]. This unique ability of depolymerases to specifically recognize and degrade EPSs and related biofilm components provides an attractive and promising tool for pathogen control [30]. On the other hand, biofilms are also known to develop within drinking lines in, e.g., poultry production systems (Maes et al., 2019), pointing towards the use of depolymerases as a management practice,

with it also assisting AMR management. An example is provided by the tail spike protein derived from φAB6 with depolymerase activity, which can significantly inhibit the formation of and degrade existing biofilms at a concentration ≥0.78 ng [31]. Moreover, such proteins have also been found to be effective in reducing *A. baumannii* adhesion on the surface of medical devices [31].

5. Novel Technologies Applied to Phage Therapy

Recently, a number of technological developments based on phage therapy have been described, in addition to the traditional therapeutic schemes mentioned so far. One application is based on the work of Ran et al. (2021), who developed a unique photodynamic antimicrobial agent (APNB) based on a cationic photosensitizer and a bacteriophage for precise bacterial eradication, also showing high efficacy against biofilm [32]. NB is a benzoxazine compound, which is a well-known DNA-binding dye with relatively low systemic toxicity and in some cases is also known for delaying tumoral growth. In this context, NB can direct selective phototoxicity in combination to phage therapy, increasing the effectiveness of the latter, which when used alone could not achieve optimal therapeutic results [33]. The combination of the dye to the phage as an antimicrobial agent allows for the real-time monitoring and evaluation of the treatment dynamics, based on the NB fluorescence. Further structural modification with, e.g., sulphur atoms provide an excellent reactive oxygen species generation ability, which could be used in combination with APNB specificity towards binding pathogenic microorganisms. Both in vitro and in vivo experiments demonstrated that APNB can effectively treat *A. baumannii* infection. However, it ought to be mentioned that *A. baumannii* recovered faster after APNB treatment compared to ampicillin and polymyxin B in mice, despite APNB having promise with regard to its application against MDRP and biofilm [34].

In terms of new technologies based on phage therapy, aerosol spray applied to both poultry and bedding material in production facilities may help prevent the horizontal transmission of pathogens. Indeed, phage-based products can be used as biological disinfectants in hatcheries, farms, transport containers, poultry processing plants and food contact surfaces. Although not trialed against *A. baumannii*, bacteriophage-based surface disinfectants, such as BacWash TM (OmniLytics Inc., Salt Lake City, UT, USA), which targets Salmonella, can be used as cleaning agents. Similarly, Ecolicide PX™, which targets *E. coli* O157:H7, has been developed to purify the skin of live animals prior to slaughter [35]. El-Gohary et al. [36] demonstrated that treating pads by spraying a bacteriophage preparation against *E. coli* could limit its spread in broilers. Similar phage therapy applications are rarely reported against *A. baumannii*; however, based on these successful examples in poultry production, it is particularly important to study and include *A. baumannii* as a therapeutical target, both as a zoonotic agent and to limit the correlated spread of AMR.

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