Comparative treatment of *Pseudomonas aeruginosa* burn wound infection using bacteriophage MB08 and antibiotics

Justin Paolo B. Abengaña¹, Irni Mark C. Gemzon¹, Jonathan Mark S. Leung¹, John Carlo A. Mamanag¹, Jose C. Nolasco Jr.¹, Ma. Sheila M. de Jesus¹, & Donna May D.C. Papa¹²*

¹Department of Biological Sciences, College of Science, ²Research Center for the Natural and Applied Sciences, University of Santo Tomas, España Boulevard, 1015 Manila, Philippines

Bacteriophage therapy is a potential alternative to antibiotics in combating multidrug resistant pathogens. The efficacy of antibiotics and bacteriophage were evaluated in the treatment of burn wound infection induced with *Pseudomonas aeruginosa*. A third degree burn wound was performed in Sprague-Dawley rats and infected with *P. aeruginosa* via topical route. The efficacy of antibiotics (Colistin and Gentamicin) topically applied daily was compared against the efficacy of *P. aeruginosa* Phage MB08 in hydrogel also applied topically but only in a single time on the burn wound. In comparison to untreated control rats, those treated with a single dose of bacteriophage showed significant reduction in the number of bacteria present in the rat’s blood and displayed a notable difference in their state of health. On the other hand, rats administered with antibiotics showed less potency in treating the burn wound infection. The results showed that Phage MB08 has therapeutic significance in treating burn wound infections in rats since a single topical application of this phage was able to rescue rats from infection caused by *P. aeruginosa* in comparison to multiple topical applications of antibiotics.

**Keywords:** *Pseudomonas aeruginosa*, bacteriophage, Colistin, Gentamicin, Sprague-Dawley

**INTRODUCTION**

Bacteriophage therapy is an important alternative to antibiotics in the current era of multidrug resistant pathogens. Since the development and extensive production of antibiotics, people have looked at them as “miracle drugs” to treat bacterial infection. Unfortunately, the escalation of antibiotic resistant bacteria is threatening the ability to combat these pathogens. This phenomenon in the modern medicine has led to renewed interest in alternative antimicrobial therapies, one of which is bacteriophage therapy. Recent studies have shown that phage therapy has the antibacterial potential to efficaciously address these antibiotic-resistant bacterial infections through its capability to invade and kill bacteria [1]. Moreover, bacteriophages are continuously utilized in the former Soviet Union.

*To whom correspondence should be addressed*  
E-mail: donnamaydelacruz@yahoo.com
and other countries in Eastern Europe administered as cocktails in different route of applications.

Despite its obvious efficacy in curing antibiotic-resistant infections it is still considered experimental [2]. In the Philippines, there is lack of knowledge of what phage therapy is and what it is all about, because it is not as prevalent as antibiotics, and very few studies have been done.

The Philippines have had problems with people purchasing antibiotics without a prescription. This sort of self-treatment is a serious threat to both the health of the individual self-medicating and to general public, because taking a course of antibiotics improperly can encourage the evolution of bacteria resistant to that medication. Many studies have proven that bacteriophage therapy can greatly help in the field of medicine as they were able to treat antibiotic resistant pathogenic bacteria that was induced in animal models [3].

_Pseudomonas aeruginosa_ is an opportunistic gram-negative bacillus that seldom causes infections in healthy individuals however can cause serious infections in immunocompromised hosts, including patients with burn wounds. Bacterial infection of the burn wound such as _P. aeruginosa_ is one of the most serious complications of burn injury. The ability of _P. aeruginosa_ to survive under different environmental conditions, combined with its inherent resistance to several antibiotics, allows it to colonize and proliferate within the burned tissues. This localized proliferation may lead to systemic sepsis, which is often associated with a high degree of mortality. The pathogenesis of _P. aeruginosa_ had been successfully examined in the non-lethally burned mouse model developed by Stieritz and Holder [4]. Since only a few antibiotics are effective against the antibiotic resistant _P. aeruginosa_, it is considered to be highly pathogenic.

In this study, by using Sprague-Dawley rats in a burn wound model, the efficacy of bacteriophage therapy was determined in comparison with the leading antibiotics being used against _P. aeruginosa_.

**METHODOLOGY**

**Bacterial organism.** Clinical isolates of _P. aeruginosa_ (ATCC 27853-1) was obtained from A/Prof. Ma. Sheila de Jesus, M.Sc., Department of Biology, College of Science, University of Santo Tomas, Manila, Philippines, and maintained in Tryptic Soy Agar (TSA) slants at 4°C.

**Bacteriophage selection.** A total of 16 phages were used for the selection of activity against _P. aeruginosa_. Among the 16 phages provided, only Phage MB08 exhibited plaque formation specific for _P. aeruginosa_. A loopful of _P. aeruginosa_ was inoculated into 10 mL of Tryptic Soy Broth (TSB) and was incubated for 12 h at 37°C. This broth culture served as the host organism. Tubes containing 2 mL of soft agar were melted in a water bath and were kept at 42°C. Briefly, 0.5 mL of the host organism and 0.1 mL of the phage filtrate were added to the tube containing soft agar. The contents of the tube were poured onto the surface of TSA plates. After the soft agar layer solidified, the plates were incubated at 37°C for 24 h. Plates that exhibited plaque formation were selected for use in the treatment of burn wound infection in the animal model part of the experiment.

**Production of high titer phage.** A loopful of _P. aeruginosa_ was inoculated into 10 mL of TSB for 5 h at 37°C. Previous plates with phage plaques were used; a 5 mm square block of agar containing plaques was cut using an inoculating needle and transferred to the tube containing the 5-h old host culture and was incubated for 24 h at 37°C. Series of filtration was performed using a 0.45 μm and 0.22 μm Acrodisc® syringe filter.
Enumeration of bacteriophages. Enumeration of bacteriophages was carried out by making serial dilutions of the *P. aeruginosa* phage filtrates. Each dilution of phage suspension was subjected to the agar layer method described previously. Plates were incubated at 37°C for 24 h. After incubation, the number of plaques were counted on the appropriate dilutions. The dilution containing 10⁸ and 10⁹ PFU/mL were used for the animal model part of the experiment.

Animals. Sprague-Dawley female rats (7–10 weeks old) were obtained from Plegaria Biological & Zoological Supply, Binangonan, Rizal. All animals were given food and water ad libitum. The animal study was conducted following protocols approved by the Institutional Animal Care and Use Committee (see attached Animal Research Permit). All the experiments were conducted in triplicate.

Burn wound model. A third degree burn wound infection model was developed in rats using *P. aeruginosa* following the method of Busch, *et al.* [5] and of Kumari *et al.* [6]. The hair on the rats’ backs were shaved and the skin was washed with 95% ethanol. Rats were anesthetized with ether fumes and brass blocks (2.54 × 2.54 × 15.25 cm) were preheated by immersion in boiling water. For each rat, two blocks of 1-in² surface area were applied to opposing sides of the exposed dorsal skin to make a 2-in² total burn area. The brass block was compressed for 15 s to deliver a full-thickness skin burn. Immediately after the burn, all rats were injected with 0.8 mL of sterile physiological saline for fluid replacement to prevent overt shock and acetaminophen (0.25 mg/mL) was given as a post burn analgesic in drinking water. Bacterial inoculum was prepared by inoculating *P. aeruginosa* in TSB, incubating at 37°C for 24 h followed by repeated centrifugation (10,000 rpm for 10 min) and washing, then finally resuspending in normal saline. To determine the LD₉₀ (Lethal dose causing 100% mortality) value of *P. aeruginosa* culture, doses ranging from 10⁴ to 10¹⁰ CFU/mL were evenly applied topically on the burn site, after a waiting period of 30 min. A wound dressing was applied to the rats in order for the wound to stay moist and prevent it from infection of other bacteria. All the burned rats inoculated with bacteria were scored for their state of health on a scale of 5 to 0, based on progressive disease state reflected by several clinical signs. A normal and unremarkable condition was scored as 5; slight illness, defined lethargy and ruffled fur, was scored as 4; moderate illness, defined as severe lethargy, ruffled fur, and hunched back, was scored as 3; severe illness, with the above signs plus exudative accumulation around partially closed eyes, was scored as 2; a moribund state was scored as 1; and death was scored as 0. The dose giving 100% lethality within 72–96 h was taken as the optimum LD₉₀.

Antibiotic and bacteriophage treatment. The efficacy of phage and antibiotics was evaluated by using six groups of rats (three rats each). A full-thickness skin burn was induced in all groups (except group 1) and was challenged with a LD₉₀ of *P. aeruginosa* culture directly on the burn site as described earlier. In group 1, rats were left without a burn wound and were used as negative controls. In group 2, all wounded rats were applied with the bacterial inoculum and were left untreated, and acted as positive controls. In groups 3 and 4, rats were burned, infected and treated with a daily topical application of Colistin and Gentamicin, respectively. In groups 5 and 6, burned and infected rats were treated with a single topical application of bacteriophage mixed with hydrogel at 1⁸ (low titer) and 1⁹ (high titer), respectively.

Enumeration of bacteria present in blood. After 96 h post-infection, blood samples were collected from each rat through tail clipping. A clean pair of sharp surgical scissors was used and not more than 3 millimeters of the tail was...
snipped. Two drops of blood, approximately 100 μL, from the tail of each rat was allowed to drop on duplicate plates, and spread plate technique was performed after. All plates were incubated for 24 h at 37°C and colonies on each plate were counted.

Statistical analysis. Student’s t test for direct mean comparison was performed with GraphPad Software (GraphPad Software, Inc., San Diego, California, USA). Difference with \( P \leq 0.05 \) was considered statistically significant.

RESULTS AND DISCUSSION

Among the 16 phage filtrates that were obtained, only 1 showed lytic capability against \( P. \) aeruginosa ATCC 27853-1 (Fig. 1). This phage strain was a previously isolated bacteriophage from a sewage treatment plant in the Philippines [7] known as Phage MB08. Its average plaque size ranges from 2.0–3.0 mm in diameter.

From this filtrate, a high titer phage was produced with a final concentration of \( 3.13 \times 10^{11} \) PFU/mL.

For the testing of lethality of \( P. \) aeruginosa, Sprague-Dawley rats induced with a third degree burn wound and inoculated with \( 10^8 \) CFU/mL died within 72–96 h (Fig. 3). This dose was recorded as LD\(_{100}\) and was used for the experiment.

The efficacy of antibiotics (Colistin and Gentamicin) topically applied daily was compared with the efficacy of Phage MB08 in hydrogel also applied topically but only in a single time on the burn wound. In comparison to untreated control rats, those treated with a single dose of bacteriophage showed significant reduction in the number of bacteria present in the rat’s blood (Figs. 3 and 4). On the other hand, rats administered with antibiotics showed less potency in treating the burn wound infection.

Figure 1. Plaques of bacteriophage on a lawn of \( P. \) aeruginosa

Figure 2. Lethality of \( P. \) aeruginosa at dose \( 10^8 \) CFU/mL in a burn wound model. A score of 5 indicates normal health, while 0 indicates death.

Figure 3. Bacteriophage present in 100 μL of blood in each animal group after 96 h post infection (date presented in mean)
Bacteriophage therapy of *Pseudomonas aeruginosa*

The amount of bacteria present in blood of rats treated with $10^8$ PFU/mL of phage was found to be significantly different from those obtained with Colistin and with Gentamicin. The same behavior was made from rats treated with $10^8$ PFU/mL of phage.

**CONCLUSIONS**

The number of bacteria present in the blood of rats treated with bacteriophage was found to be significantly lower compared to rats treated with antibiotics. The single topical application of bacteriophage used was more effective than the multiple applications of antibiotics in treating burn wound infections caused by *Pseudomonas aeruginosa* and other skin microbial flora.

In order to have a further understanding on bacteriophage therapy, this study recommends future researchers to identify and treat other organisms that could also be a common cause of bacterial burn wound infection and explore the potential of bacteriophage therapy in treating other forms of bacterial infection. Furthermore, comparing the efficacy of antibiotics and bacteriophage therapy through different kinds of administration would also be compelling.

**REFERENCES**


