



EVALUATION OF HEMATOLOGICAL AND BLOOD BIOCHEMICAL INDICES IN CULTURED NILE TILAPIA (*Oreochromis niloticus*) AS AFFECTED BY USING PHAGE THERAPY AGAINST *Pseudomonas aeruginosa*

Nahed A. EL-Wafai^{1*}, Sara A.T. EL-Ged¹, Victor S. Bedrous¹, Mamdouh A.A. Mousa², Ashwag Shami³, Khalid M. Al Syaad⁴, Majed A. Bajaber⁵, Ahmed Ezzat Ahmed^{4,6}, Mada F. Ashkan⁷, Afaf S. Alwabri⁷, Amani Osman Shakak^{7,8}, Fatimah S. Alqahtani¹⁰, Eman A. Beyari¹⁰, Behairy A. Akl¹

¹Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

²Central Laboratory for Aquaculture Research, Abbassa, Abu-Hammad, Sharqia, Egypt

³Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia

⁴Biology Department, College of Science, King Khalid University, PO Box 9004, Abha 61413, Saudi Arabia

⁵Chemistry Department, College of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

⁶Prince Sultan Bin-Abdul-Aziz Center for Environment and Tourism Studies and Researches, King Khalid University, PO Box 960, Abha 61421, Saudi Arabia

⁷Biological Sciences Department, College of Science and Arts, King Abdulaziz University, Rabigh 21911, Saudi Arabia

⁸Faculty of Medical Laboratory Sciences, University of Shendi, Shendi P.O. Box 142, Sudan

⁹Department of Biology, Faculty of Sciences, University of Bisha, P.O. Box: 551, Bisha 61922, Saudi Arabia

¹⁰Department of Biological Sciences, Microbiology, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia

*Corresponding author: nahedelwafai76@yahoo.com; nahedelwafai19@gmail.com

Abstract

Pseudomonas spp. causes significant losses in aquaculture, consecutive use of antibiotics, and reveals bacterial resistance; therefore, therapeutic bacteriophages, commonly called phages, are a promising potential alternative to antibiotics in the management of bacterial infections of a wide range of organisms, including cultured fish. The novelty of current work is represented in examining the lytic activity of four phages and their combination compared to the antibiotic streptomycin on *Pseudomonas aeruginosa*-infected Nile tilapia (*Oreochromis niloticus*) while measuring the hematological and blood biochemical parameters as a response for phage therapy. This study evaluated the *in vitro* killing curve for each phage using a growth curve that measured the OD₆₀₀ after a single phage suspension was combined with the host *P. aeruginosa*, considered the best multiplicity of infection (MOI) for each phage. A144 healthy fish were acclimatized in the laboratory and divided into six groups: control, *P. aeruginosa*-infected fish, streptomycin, phage Ps1, Ps2, both (Ps1 and Ps2), were added to the T3, T4, T5, and T6 groups, respectively. Our findings demonstrated that *P. aeruginosa* infection caused surface body hemorrhages, tail and fin rot, irritated skin, superficial ulcers, and 100% mortality through 14 days. *P. aeruginosa* caused a reduction in hemoglobin (Hb), red blood cells (RBCs), platelet number (PLt), and platelet crit (PCT) count, protein, albumin, and A/G ratio; however, an increase in hematocrit (Hct), red cell distribution width (RDW), PDW, MPV compared to other groups after three days of infection and the effects increased after 12 days post-infection. The fish vaccinated with P1 (T4) and P1+P2 (T6) showed enhanced levels of Hb, RBCs, PLt, PCT, protein, albumin and decreased levels of RDW, PDW, MPV, and liver and kidney enzymes with enhanced contents more than streptomycin and closer to the control group. The biochemical markers recorded significant changes indicating liver and kidney impairments due to the infection with *P. aeruginosa*. It can be concluded that P1 and P1+P2 combinations could be used as therapy in *Pseudomonas*-infected fish to enhance their blood parameters and performance.

Key words: bacteriophages, *Pseudomonas aeruginosa*, phage therapy, blood parameters, Nile tilapia

Pseudomonas spp. causes the loss of the most economically valuable fish, Nile tilapia *Oreochromis niloticus* (El-Saadony et al., 2022 a). Inadequate water quality, parasites, and stress increase ammonia levels because of the high stocking density and feeding (Abd El-Hack et al., 2022 a). Diseases induced by pathogenic bacteria like *Aeromonas* spp., *Vibrio* spp., *Pseudomonas* spp., and *Flavobacterium* spp., are one of the hurdles to the growth

of cage aquaculture in open waters (El-Saadony et al., 2021; Manna et al., 2023), which leads to the spread of resistant strains in the environment (Catho et al., 2021).

Pseudomonas aeruginosa is a Gram-negative, opportunistic bacterial pathogen and one of the leading causes of nosocomial infections (Crone et al., 2020). *Pseudomonas aeruginosa* is a part of normal fish microbiota;

however, under stressful conditions such as malnutrition and overcrowding, the bacteria have become highly opportunistic and pathogenic, causing serious illness, including hemorrhagic septicemia, gill necrosis, abdominal distension, splenomegaly, friable liver, and congested kidney (Ardura et al., 2013).

Pseudomonas isolates had the most excellent minimal inhibitory concentration (MIC) and the highest rate of antibiotic resistance (Iliev et al., 2015). Bacteriophages are one of the most potent alternatives to antibiotic usage (Golkar et al., 2014). These naturally occurring bacterial viruses can destroy certain bacteria without affecting the rest of the flora.

Most known phages are tailed viruses belonging to the *Caudovirales* order. They are built of a capsid with nucleic acid (either DNA or RNA) and a tail that may vary in size. The capsid consists of proteins or lipoproteins that protect the genetic material of the phage, while the tail recognizes the bacterial host via specific receptors located at the tail fibers (Doss et al., 2017).

In aquaculture, phages of different families can be encountered for phage therapy, mostly *Siphoviridae*, *Myoviridae*, or *Podoviridae* (Zhang et al., 2018). Phages reduce pathogens and enhance aquaculture (Hodgson, 2013; Housby and Mann, 2009; Ly-Chatain, 2014). Phages can have an excellent therapeutic impact as phage proliferation by auto “dosing” results in a bactericidal effect (Abedon and Thomas-Abedon, 2010).

Bacteriophages are bacterial viruses that attack a particular species or strain of bacteria. An extreme specification of the phage host range minimizes the usage of a single phage kind as a biological control against multi-drug resistant (MDR) bacteria. Additionally, this could hasten the appearance of bacterial mutants resistant to phages (Hyman and Abedon, 2010; Yen et al., 2017). Hematological analyses provide knowledge about fish health status (Jamalzadeh et al., 2009). Variations in hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), differentiation of leukocytes, and the size of RBCs and WBCs are critical findings in diagnosis (Summarwar and Verma, 2012). A cocktail of more than one phage was recommended for use as a biocontrol against fish diseases to expand the host range of phages (Kelly et al., 2011; Mapes et al., 2016; Wright et al., 2009).

No studies investigated the lytic activity of four phages and their combinations in one study; in this study, we recovered four lytic bacteriophages infecting *P. aeruginosa* from agricultural wastewater and soil and then identified their shapes and infection cycles. This investigation assessed four phages (Ps1, Ps2, Ps3, and Ps4) for their ability to inhibit *P. aeruginosa* growth *in vitro*. Then, an assessment of blood parameters in Nile tilapia fish (*O. niloticus*) was performed *in vivo* to study the effect of intraperitoneal phage therapy (Ps1 and Ps2) against multiple drug-resistant *P. aeruginosa* to various antibiotic classes compared with using streptomycin, which strain was sensitive to antibiotics.

Material and methods

Pseudomonas isolation, screening, identification and challenge

Pseudomonas isolates were isolated from water, which were collected randomly from different regions. The water samples were collected, mixed, and transferred to the microbiology laboratory. Serial dilutions from soil samples were prepared as follows: 10 g water was homogenized in 90 mL peptone water to obtain 10^{-1} dilution, 1 mL from the previous dilution was added to 9 mL of peptone water to get 10^{-2} dilution, and serial dilutions up to 10^{-7} . 1 mL of each dilution was transferred into separate sterile petri dishes. Sterile cetrimide agar containing 20 g of pancreatic digest of gelatin, 10 g of potassium sulfate, 1.4 g of magnesium chloride, 0.3 g of acetyltrimethylammonium bromide, 10 mL of glycerin and 13.6 g of agar was dissolved in one liter of water and poured aseptically into the petri dishes, and the culture plates were incubated at 37° C for 2 days. To obtain distinct colonies, bacteria were further sub-cultured on cetrimide agar. Presumptively, blue-green pigment colonies were aseptically picked and stored on nutrient agar slants for further biochemical characterization (Adesoji et al., 2016).

The *Pseudomonas* isolates were screened based on their resistance to antibiotics ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (300 µg), and ampicillin (10 µg) (Abtek Biologicals Ltd). The antibiotics susceptibility profiles were determined by agar diffusion technique on Mueller Hinton agar (MHA). An inoculum suspension was prepared and standardized to match the 0.5 McFarland's standard which corresponds to approximately 1.5×10^8 cfu/mL. Using sterile swab sticks, cells from the inoculum suspension were evenly spread on the MHA and allowed to dry. Plates were aseptically impregnated with antibiotic sensitivity discs and incubated at 37°C overnight. Zones of inhibition around the antibiotic discs were measured, and the lengths were categorized as resistant, intermediate and sensitive, following the Clinical Laboratory Standards Institute guidelines for each bacteria isolate (CLSI, 2021). Multi-drug resistant bacteria were selected based on their resistance to ≥ 3 classes of antibiotics. The isolates coded as AM1, AM2, ..., AM35; *Pseudomonas* AM16 showed MDR to all antibiotics as follows, 100% resistance to each of cefuroxime and nitrofurantoin. This was followed by 90% resistance to each of amoxicillin/clavulanate, ampicillin and ceftazidime respectively. The isolates demonstrated the least (60%) resistance to gentamicin compared to other isolates.

Presumptive *P. aeruginosa* isolates were further subjected to biochemical tests such as Gram staining, catalase and oxidase tests, reactions on triple sugar iron agar and motility test to further confirm the identity of the isolates. The tests results were then compared to the reference standards in the Bergey's Manual. The results of morphological and biochemical tests indicated that *Pseu-*

domonas AM16 is similar to *Pseudomonas aeruginosa*. Further identification was conducted by MALDI-TOF spectroscopy; the selected isolate *Pseudomonas aeruginosa* AM16 was identified as *Pseudomonas aeruginosa* DSM 939.

P. aeruginosa was inoculated into nutrient agar and incubated at 37°C for 24 h. Bacterial colonies were collected in sterile saline (0.85% NaCl). 1 ml of saline contained 3×10^6 CFU/ml (LD50) using a McFarland standard tube, according to Elsayed et al. (2018). Fish in all groups were intraperitoneally (IP) challenged with 0.2 ml/fish of a bacterial suspension of *P. aeruginosa*.

In vitro killing curves in bacteriophages

P. aeruginosa inactivation was determined individually for phages (Ps1, Ps2, Ps3, and Ps4) isolated and identified in the Agricultural Microbiology Department, Faculty of Agriculture, Zagazig University laboratory. Different multiplicities of infection (MOIs) (0.01, 0.1, 1.0, and 10.0) against *P. aeruginosa* were selected to determine the best MOI for each phage. As a result, a killing curve for each phage was produced. A growth curve instrument was used to measure the OD₆₀₀ after a single phage suspension was combined with the host *P. aeruginosa* (10^8 CFU/ml) at the ideal MOI (Bio Screen C, 110001-892, Japan). Data were gathered for 6 hours at 37°C at 15-minute intervals. The double agar layer assay was used to assess the initial titer of each phage for all phage infection techniques (Pereira et al., 2011). Five flasks were used for each assay, each containing 100 ml inoculated with 1 ml of *P. aeruginosa* (10^8 CFU/ml). One was left as a control, and the others were vaccinated separately with one of the following phages (Ps1, Ps2, Ps3, and Ps4). Flasks were incubated in a shaking incubator at 37°C for 6 h.

In vitro phage cocktail application

The optimal MOI (0.01) was selected based on the killing curves of each phage against *P. aeruginosa* at different MOIs (0.01, 0.1, 1, and 10). The phage cocktails of 2, 3, or 4 phages were calculated within 80 hours compared to the following bacterial control. Each phage was utilized at the same density (10^6 PFU/ml) (Donati et al., 2021): 1: (Ps1 and Ps2), 2: (Ps1 and Ps3), 3: (Ps1 and Ps4), 4: (Ps2 and Ps3), 5: (Ps2 and Ps4), 6: (Ps3 and Ps4), 7: (Ps1, Ps2 and Ps3), 8: (Ps1, Ps2 and Ps4), 9: (Ps1, Ps3 and Ps4), 10: (Ps2, Ps3 and Ps4), 11: (Ps1, Ps2, Ps3 and Ps4).

In vivo experimental design

A group of 144 fish was judged healthy from its overall normal morphology, feeding behavior, active breathing, active movement, brightness, and clear eyes. Nile tilapia (*Oreochromis niloticus*) fish with an initial weight of 60 g were purchased from the Fish Research Central Lab., Ministry of Agriculture, Egypt. Fish were acclimated in glass aquaria containing dechlorinated water for two weeks. Water parameters were checked weekly.

The quality of water was monitored and stabilized; the sodium, potassium, magnesium, and calcium levels in the water were adjusted to 0.4, 0.05, 0.6, and 0.8 mmol L⁻¹, respectively. At a temperature of $25.4 \pm 0.3^\circ\text{C}$, and a dissolved oxygen level of 7.30 ± 0.13 mg/l, these levels were within the recommended ranges for the culture of Nile tilapia, a pH of 7.90 ± 0.06 , a total hardness level of 132 ± 4.4 mg CaCO₃/L⁻¹, ammonia (NH₃) from 0.03 to 0.038 mg/L, total salinity of 23.41 ppt, total ammonia nitrogen 0.004 ± 0.001 g/l, and a photoperiod regime (12:12 h light: dark) (Saad et al., 2022).

After the acclimation period, 144 fish were taken out of the tank and divided into six treatment groups, each with three replicates (9 fish each) in a 60-liter glass aquarium that was supplied with air by an airstone using an air pump; the experimental duration was two weeks after 14 days of acclimatization, the parameters were measured 3 and 12 days post-infection. The first group was the control. The second group was injected with *P. aeruginosa* (6×10^6 CFU/fish in 1 mL of saline). In contrast, streptomycin, phage Ps1, Ps2, both (Ps1 and Ps2), were added to the T3, T4, T5, and T6 groups, respectively.

Blood samples and analyses

Blood samples were collected from the caudal vein of non-anesthetized fish (9 from each group) using a sterile syringe on the 3rd and 12th days of the trial. The collected blood was divided into two sets of Eppendorf tubes. Sodium heparin was added to the first set as an anticoagulant for hematological experiments, hemoglobin (Hb), red blood cell counting (RBCs), and hematocrit (Hct). The second set was left without anticoagulant to clot at 4°C and centrifuged at 5000 rpm for 10 minutes at room temperature to collect the serum, which was stored at -20°C for biochemical analyses. Hemoglobin (Hb, g/dl) concentration was estimated colorimetrically following Blaxhall and Daisley (1973). RBCs, $10^6/\text{mm}^3$, were assigned according to Dacie and Lewis (1984).

A capillary hematocrit tube was used to calculate the hematocrit value (Hct,%) (Rehulka, 2000) using the following equations (Lewis et al., 2006):

$$\begin{aligned} MCV (\text{fl}) &= \text{Hct} (\%) \times 10/\text{RBC} (10^6 \mu\text{L}^{-1}), MCH (\text{pg}) = \\ &= (\text{Hb} (\text{g dL}^{-1}) \times 10)/\text{RBC} (10^6 \mu\text{L}^{-1}), MCHC (\%) = \\ &= (\text{Hb} (\text{g dL}^{-1}) \times 100)/\text{Hct} (\%). \end{aligned}$$

Red blood cell distribution width (RDW) is now integral to automated complete blood count (CBC) analysis and is accessible on all automated hematology analyzers. Platelet count (PLt) and platelet indices (mean platelet volume, MPV; platelet distribution width, PDW; and platelet crit, PCT analysis) were done by the automated hematology flow cytometry-analyzer, Technicon-H, according to Lombarts et al. (1986). WBC, $10^3/\text{mm}^3$, were indirectly counted after McKnight (1966).

Differential leukocytes (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) were examined with May-Grunwald-Giemsa-stained blood smears. Eve-

ry slide was tested under oil immersion at 100x magnification. For every slide, 100 leukocytes were characterized as neutrophils, lymphocytes, monocytes, eosinophils, or basophils. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated colorimetrically following Reitmann (1957). Urea was estimated following Patton and Crouch (1977). Creatinine was calculated after Kaplan and Glucose (1984). Serum total protein was assessed colorimetrically, according to Henry (1964). Albumin was evaluated colorimetrically following Wotton and Freeman (Wotton, 1982). Globulin was estimated by subtracting albumin value from total protein. The bilirubin was determined according to El-Houseiny et al. (2021).

Statistical analysis

According to Petrie and Watson (2013), the data were statistically analyzed using a one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test to find any significant differences between the treatments ($P \leq 0.05$). All results were recorded as mean $\pm$ standard error of the mean.

Results

Estimation of the optimal multiplicity of infection (MOI)

Four phages, namely, Ps1, Ps2, Ps3, and Ps4 were selected to determine the multiplicity of infection against *P. aeruginosa* using four different rates of MOI (0.01, 0.01, 1, and 10) at OD_{600} . The killing histograms are shown in Figure 1 (a–d). It can be shown that these phages were

very potent against *P. aeruginosa* at four MOIs. However, the effectiveness of these phages loaded at an MOI of 0.01 was more powerful than the other MOIs since it resulted in the most nominal bacterial growth compared to MOIs of 0.1, 1.0, and 10.0. Infectivity lasted until the end of 6 h of incubation at 37°C. When infected at lower MOI (0.1 and 0.01), the bacterial density (OD_{600}) significantly dropped Figure 1. On the other hand, OD_{600} increased after 105, 135, 150, and 150 min for phages Ps1, Ps2, Ps3, and Ps4, respectively, at MOI 10, Ps1, Ps2, Ps3, and Ps4 at four different MOIs: 10, 1, 0.1, and 0.01 for 6 h. Each point shows the average and standard deviations from three replicate studies.

In vitro phage cocktail application

As shown in Figure 1, cocktails containing two, three, and four phages were employed to investigate their bactericidal effects against *P. aeruginosa* cells at an MOI of 0.01. The bacterial killing curves of the two sets of cocktails containing three or four phages, as shown in Figure 2 b, resulted in no significant dominance in controlling bacterial growth compared with the cocktails containing only two phages. In contrast, the absorbance at 600 nm of the bacterial growth curve reached 0.33 after 15 h (Figure 2). Since a higher inactivation of bacterial growth was observed by the cocktails containing phages (Ps1+Ps2), they were highly efficient (Figure 2 a, c). The optical bacterial growth density decreased to less than 0.3 (OD_{600}) after the first 30 h of incubation and remained as it was throughout the end (80 h). However, the optical density of the bacterial growth was detected to be 0.05 (OD_{600}) and lasted for 80 h when the cocktail was composed of Ps1 and Ps2 (Figure 2 a).

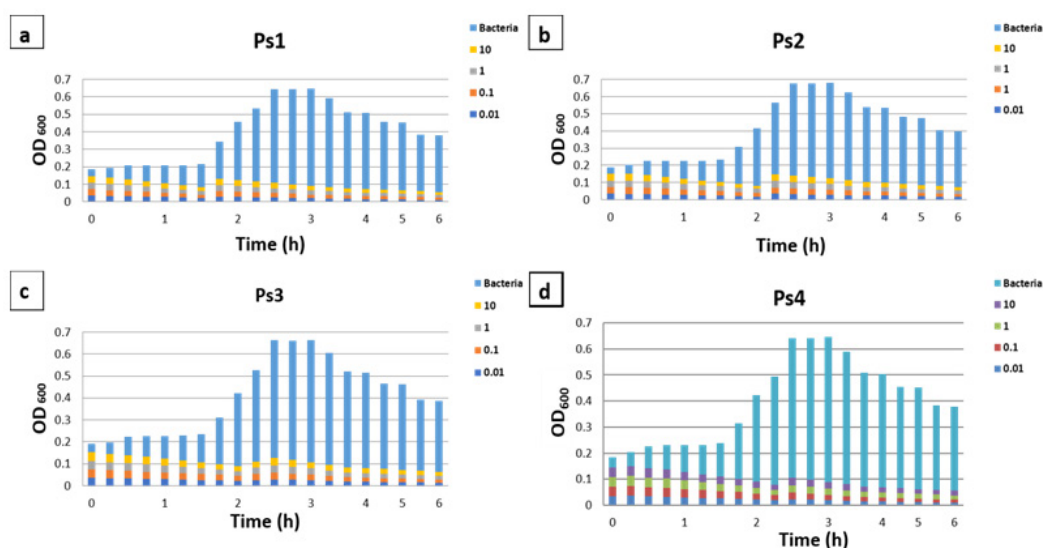


Figure 1. *In vitro* killing histograms of *P. aeruginosa* by four phages (Ps1, Ps2, Ps3, and Ps4) at four different MOIs (10, 1, 0.1, and 0.01) for 6 h

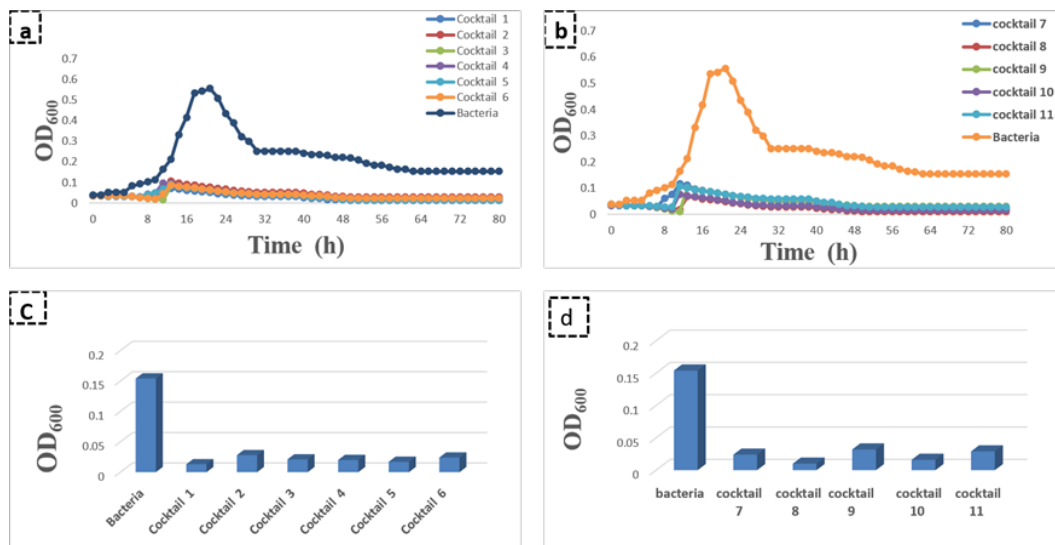


Figure 2. *In vitro* killing curves of *P. aeruginosa* (a and b) – The final OD₆₀₀ of the host bacteria by 11 cocktails phages at MOI of 0.01 for 80 h (c and d)

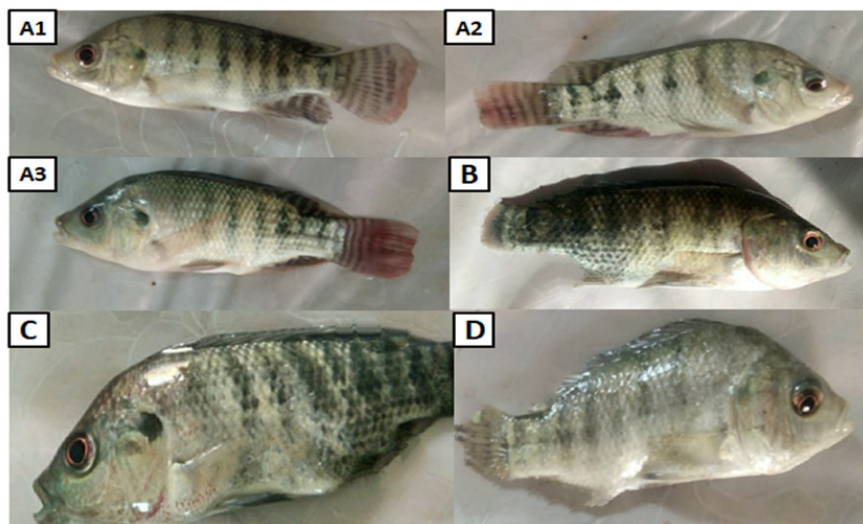


Figure 3. *P. aeruginosa* injections into fish both with and without phages (Ps1 and Ps2) or streptomycin demonstrated: A1 – fish without any injection (Control), A2 – fish injected with Ps1 and Ps2, A3 – fish injected with *P. aeruginosa* and streptomycin. B – fish injected with *P. aeruginosa* showed tail and fin rot and ulcerative skin. C – fish injected with *P. aeruginosa* showed bruises and superficial bleeding on the skin. D – fish injected with *P. aeruginosa* had tail and fin rot as well as skin viscosity

The antibacterial activity of phages on *P. aeruginosa*

In this work, two *P. aeruginosa* phages (Ps1 and Ps2) were employed, as well as the antibiotic streptomycin (10 µg), due to *P. aeruginosa*'s sensitivity to this antibiotic. Some symptoms were detected in *Oreochromis niloticus* fish injected with Ps1 and Ps2 and streptomycin, as shown in Figure 3. These symptoms were hemorrhages on the body's surface, tail and fin rot, ulcerative skin, skin viscosity, and superficial ulcers (Figure 3, B, C and D). The healthy fish received no treatment and were injected with either one phage or a cocktail of both phages (A1, A2, and A3).

Blood parameters

Data in Table 1 revealed a significant reduction

($P < 0.05$) in Hb and RBCs. At the same time, Hct was increased in *P. aeruginosa* infected *O. niloticus* (T2) in contrast to the control (T1) and other treated groups (T3, T4 and T5). However, MCV and RDW increased, but MCH and MCHC decreased. Streptomycin or phage treatment resulted in significant increases in Hb and RBCs and a decrease in Hct compared to *P. aeruginosa*-infected fish, but still lower than the control. The hematological parameters decreased more significantly after 12 days of *P. aeruginosa* infection. These parameters were improved in the fish group that received phage Ps1 and Ps 1+Ps 2 (T4 and T6) better than in (T3 or T5) but still less than in control.

Table 1. Hematological differences in Nile fish injected with *Pseudomonas aeruginosa*, streptomycin, and two phages after 3 and 12 days of infection

Treatments	Parameters	Hb (g/dl)	RBC ($10^6 \mu\text{l}^{-1}$)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (%)	RDW (%)
After 3 days								
T1		5.83 $\pm$ 0.03 a	2.63 $\pm$ 0.01 a	25.00 $\pm$ 0.23 c	91.14 $\pm$ 0.11 i	22.15 $\pm$ 0.09 d	23.34 $\pm$ 0.23 c	14.97 $\pm$ 0.12 de
T2		2.17 $\pm$ 0.02 h	1.38 $\pm$ 0.01 g	31.23 $\pm$ 0.15 a	173.92 $\pm$ 0.73 b	15.70 $\pm$ 0.14 g	6.94 $\pm$ 0.03 f	28.57 $\pm$ 0.43 a
T3		4.70 $\pm$ 0.06 d	1.94 $\pm$ 0.01 bc	18.98 $\pm$ 0.21 de	123.50 $\pm$ 0.56 e	24.18 $\pm$ 0.19 c	24.77 $\pm$ 54 abc	17.73 $\pm$ 0.92 b
T4		5.07 $\pm$ 0.17 c	1.96 $\pm$ 0.02 b	19.62 $\pm$ 0.06 de	122.47 $\pm$ 1.08 e	25.85 $\pm$ 0.87 a	25.83 $\pm$ 0.84 a	14.93 $\pm$ 0.27 de
T5		4.20 $\pm$ 0.06 f	1.72 $\pm$ 0.01 e	16.90 $\pm$ 0.12 f	139.81 $\pm$ 0.72 c	24.47 $\pm$ 0.21 b	24.86 $\pm$ 0.45 ab	12.43 $\pm$ 0.13 g
T6		4.40 $\pm$ 0.12 ef	1.78 $\pm$ 0.02 d	18.77 $\pm$ 0.2 e	134.61 $\pm$ 1.34 d	24.67 $\pm$ 0.52 b	23.45 $\pm$ 0.84 bcd	13.13 $\pm$ 0.03 fg
After 12 days								
T1		5.45 $\pm$ 0.03 b	2.64 $\pm$ 0.02 a	24.67 $\pm$ 0.88 c	93.46 $\pm$ 3.61 hi	20.66 $\pm$ 0.23 e	22.16 $\pm$ 0.70 d	13.13 $\pm$ 0.45 fg
T2		2.12 $\pm$ 0.01 h	1.41 $\pm$ 0.01 g	27.77 $\pm$ 0.09 b	196.97 $\pm$ 1.66 a	15.04 $\pm$ 0.07 g	7.64 $\pm$ 0.04 f	28.47 $\pm$ 0.39 a
T3		2.89 $\pm$ 0.09 g	1.59 $\pm$ 0.01 f	15.51 $\pm$ 0.29 f	97.35 $\pm$ 2.35 gh	18.18 $\pm$ 0.15 f	18.69 $\pm$ 0.38 e	15.57 $\pm$ 0.35 cd
T4		4.91 $\pm$ 0.01 c	1.97 $\pm$ 0.01 b	19.54 $\pm$ 0.09 de	99.17 $\pm$ 0.29 fg	24.92 $\pm$ 0.07 ab	25.13 $\pm$ 0.07 a	14.17 $\pm$ 0.17 ef
T5		4.20 $\pm$ 0.03 f	1.74 $\pm$ 0.01 e	16.27 $\pm$ 0.18 fg	93.67 $\pm$ 1.01 hi	24.20 $\pm$ 0.01 bc	25.84 $\pm$ 0.29 a	13.87 $\pm$ 0.34 ef
T6		4.46 $\pm$ 0.03 e	1.92 $\pm$ 0.02 c	19.84 $\pm$ 0.03 d	103.38 $\pm$ 1.29 f	23.24 $\pm$ 0.29 c	22.47 $\pm$ 0.15 d	16.43 $\pm$ 0.41 c

Means $\pm$ SE of 9 fish per group with three replicates are used to express data. Means in the same column with the same alphabet do not differ significantly ($P < 0.05$). Red blood cell count (RBC), hemoglobin (Hb), hematocrit value (Hct), corpuscular volume mean (MCV), corpuscular hemoglobin mean (MCH), corpuscular hemoglobin concentration mean (MCHC), and red blood cell distribution width (RDW).

Table 2. Changes in blood platelets count (PLt) and platelet indices of Nile tilapia injected with *P. aeruginosa*, streptomycin, and/or two phages after 3 and 12 days of infection

Treatments	Platelets	Plt ($\times 10^3$ cells/ μ L)	PDW (%)	MPV (fl)	PCT (%)
After 3 days					
T1		253.33 $\pm$ 2.52 a	10.97 $\pm$ 0.32 f	10.20 $\pm$ 0.20 d	0.26 $\pm$ 0.01 b
T2		115.00 $\pm$ 2.65 g	12.00 $\pm$ 0.90 de	12.37 $\pm$ 1.07 c	0.14 $\pm$ 0.02 f
T3		176.67 $\pm$ 2.52 d	11.23 $\pm$ 0.06 e	10.37 $\pm$ 0.12 d	0.18 $\pm$ 0.01 e
T4		185.67 $\pm$ 1.15 c	11.53 $\pm$ 0.21 e	10.53 $\pm$ 0.81 d	0.19 $\pm$ 0.02 e
T5		170.33 $\pm$ 1.53 e	11.30 $\pm$ 0.10 e	10.53 $\pm$ 0.06 d	0.18 $\pm$ 0.00 e
T6		172.33 $\pm$ 2.52 e	11.03 $\pm$ 0.85 f	10.53 $\pm$.06 d	0.18 $\pm$ 0.00 e
After 12 days					
T1		252.00 $\pm$ 1.00 a	11.20 $\pm$ 0.62 e	10.23 $\pm$ 0.15 d	0.26 $\pm$ 0.00 b
T2		72.00 $\pm$ 1.00 h	14.40 $\pm$ 0.52 a	13.70 $\pm$ 0.62 ab	0.09 $\pm$ 0.01 g
T3		185.00 $\pm$ 4.00 c	12.50 $\pm$ 0.35 d	13.30 $\pm$ 0.17 b	0.25 $\pm$ 0.02 cd
T4		196.67 $\pm$ 0.58 b	13.77 $\pm$ 0.68 b	14.47 $\pm$ 0.15 a	0.29 $\pm$ 0.01 a
T5		166.33 $\pm$ 1.53 f	13.13 $\pm$ 0.31 c	14.07 $\pm$ 0.50 ab	0.23 $\pm$ 0.01 d
T6		182.67 $\pm$ 2.52 c	13.57 $\pm$ 0.12 bc	14.30 $\pm$ 0.26 a	0.26 $\pm$ 0.01 b

Information presented as means $\pm$ SE of 9 fish per group with three replicates. Means in the column with the same alphabet do not differ significantly ($P>0.05$). Platelet distribution width (PDW), mean platelet volume (MPV), procaltitonin (PCT).

Data in Table 2 show that PLT and PCT decreased. However, PDW and MPV increased significantly in the fish group infected by *P. aeruginosa* individually (T2) and combined with streptomycin (T3) or phages. The best results next to the control group were recorded in the fish group injected with phage Ps 1 (T4). The highest values were recorded in the fish infected by *P. aeruginosa* alone (T2). Data in Table 3 showed significant increases in total leukocyte counts (WBCs) and all different types of the infected fish group (T2) in contrast to the control (T1). The whole numbers in the other groups were less than the counts in T2 but still above those in the control (T1) group. After the third day, the total counts in the fish groups treated with phages Ps 1 (T4) and Ps 2 (T5) were less than those in the fish group treated with streptomycin (T3). After the 12th day, the results fluctuated but were still above the control fish group (T1). The best results next to the control were in T4.

On the third day of infection, ALT and AST of Nile tilapia infected by *P. aeruginosa* (T2) were significantly elevated in contrast with the control (T1) (Table 4). These increases were less than those in the infected Nile tilapia that received streptomycin or phages. On the 12th day, serum ALT and AST were markedly decreased ($P<0.05$) in T2 compared to the control, while other groups showed lower but still above the control group. Moreover, urea, creatinine, total bilirubin, direct bilirubin, and indirect bilirubin were elevated in a significant way in T2 compared to the control T1 and other fish groups on the 3rd and 12th days of infection. The best results were recorded in T4 after control (T1).

The protein profile of *O. niloticus* infected by *P. aeruginosa* (T2) showed a significant decrease ($P<0.05$) in total serum protein, albumin, and A/G ratio. In addition, *O. niloticus* that received streptomycin (T3) or phages Ps1 and Ps2 (T4, T5, and T6) displayed a significant decrease in total protein, albumin, and A/G ratio compared to the control (T1) but above their values in T2 on the 3rd and 12th days (Table 5). Globulin increased significantly on the 3rd day and decreased on the 12th day. The fish in group T2 were the most affected, next to T3, T6, and T5. The best results were recorded in T4 compared to the control ones (T2).

Discussion

Pathological variables, both external and internal, can have an impact on animals' productivity (Swelum et al., 2021; Abd El-Hack et al., 2022 b; El-Saadony et al., 2022 b). The multiplicity of infections is one of the most substantial factors in treating challenging bacteria (El Zlitne, 2022). In addition, it was observed that phage-resistant bacteria might be stimulated rapidly by a huge phage load (MOI: 10) since an upper killing histogram was obtained when MOI was 10 with the four phages tested in this study (Figure 1 a, b, c, and D). The bacterial cells were inhibited more at MOIs 0.1 and 0.01 than at MOI 10 with the four phages tested. More free phage particles may quickly encourage bacterial resistance to either of the four phage infections.

Table 3. Changes in total counts of leukocytes (WBCs) and other blood types in the Nile tilapia (*O. niloticus*) blood injected with *P. aeruginosa*, streptomycin, and two phages after 3 and 12 days of infection

Leukocytes		WBCs (10^3 ul^{-1})	Neutrophils (10^3 ul^{-1})	Segmented (10^3 ul^{-1})	Band (10^3 ul^{-1})	Lymphocytes (10^3 ul^{-1})	Monocytes (10^3 ul^{-1})	Eosinophils (10^3 ul^{-1})	Basophils (10^3 ul^{-1})
Treatments									
After 3 days									
T1		12.30±0.15 g	6.33±0.33 g	5.33±0.33f g	1.00±0.00 def	3.47±0.03 fg	1.67±0.33 def	0.83±0.17 cd	0.00
T2		49.70±1.53 a	25.40±1.14 a	21.00±1.00 a	4.40±0.31 a	16.20±0.06 b	5.10±0.66 a	3.00±0.58 a	0.00
T3		22.23±1.65 c	12.10±0.26 c	10.67±0.33 c	1.77±0.12 bc	3.23±0.12 g	4.00±0.58 a	2.80±0.20 a	0.00
T4		15.57±0.09 ef	7.43±0.03 efg	5.37±0.09 fg	2.07±0.07 a	4.13±0.09 ef	2.37±0.19 cde	1.63±0.19 bc	0.00
T5		15.27±0.3 ef	7.83±0.09 ef	5.83±0.17 fg	2.00±0.12 a	3.27±0.43 fg	2.50±0.29 bc	1.67±0.33 bc	0.00
T6		21.90±0.81 c	10.33±0.33 d	8.33±0.33 d	2.00±0.00 a	6.00±0.58 d	3.23±0.27 bc	2.33±0.33 ab	0.00
After 12 days									
T1		11.53±0.23 g	6.67±0.33 fg	6.17±0.17 efg	0.50±0.29 f	3.40±3.06 fg	1.00±0.00 f	0.50±0.29 d	0.00
T2		40.37±1.03 b	16.37±0.58 b	15.00±0.58 b	1.37±0.07 cde	20.33±0.33 a	2.50±0.29 cd	1.17±0.17 cd	0.00
T3		17.00±0.21 de	7.90±0.25 ef	6.10±0.49 efg	1.80±0.26 bc	5.93±0.23 d	2.00±0.00 def	1.17±0.17 cd	0.00
T4		14.47±0.22 f	7.37±0.09 efg	5.83±0.23 fg	1.53±0.29 Bcd	4.37±0.27 e	1.33±0.33 ef	1.40±0.31 cd	0.00
T5		18.27±0.37 d	8.27±0.37 e	7.33±0.33 de	0.93±0.07 c	6.83±0.17 c	2.00±0.00 def	1.17±0.17 cd	0.00
T6		17.57±0.30 d	8.30±0.15 e	7.17±0.17 def	1.13±0.13 de	6.00±0.00 d	2.00±0.00 def	1.17±0.17 cd	0.00

Values presented as mean ± SE of 9 fish per group with three replicates. Means in the columns with the same letter are insignificant ($P>0.05$).Table 4. Changes in serum ALT, AST, urea, creatinine, and bilirubin in Nile tilapia (*O. niloticus*) injected with *P. aeruginosa*, streptomycin, and two phages after 3 and 12 days of infection

Parameters		ALT (u/l)	AST (u/l)	Urea (g/dl)	Creatinine (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)
Treatments								
After 3 days								
T1		12.00±0.58 h	14.00±0.58 f	7.57±0.15 c	0.12±0.01 f	2.79±0.01 f	0.33±0.02 f	2.46±0.03 a
T2		96.00±1.00 a	197.33±4.26 a	13.37±0.64 a	0.19±0.00 d	3.98±0.02 b	1.87±0.13 cd	2.10±0.12 b
T3		61.67±1.20 d	74.33±0.67 b	7.57±0.07 c	0.17±0.01 de	2.48±0.02 g	1.85±0.03 d	0.63±0.01 e
T4		61.00±1.73 d	75.33±1.20 b	7.73±0.07 c	0.14±0.02 e	2.44±0.04 g	0.89±0.03 e	1.53±0.01 c
T5		67.00±1.53 b	67.67±2.03 c	7.78±0.06 c	0.15±0.02 de	2.28±0.02 h	0.95±0.01 e	1.34±0.03 cd
T6		64.33±2.60 c	75.00±1.73 b	7.68±0.05 c	0.16±0.01 de	2.34±0.02 h	1.15±0.08 e	1.19±0.06 d
After 12 days								
T1		11.00±0.58 h	12.00±0.58 f	7.27±0.13 c	0.18±0.01 d	3.16±0.02 e	2.01±0.06 cd	1.16±0.06 d
T2		8.33±0.33 i	9.67±0.33 g	11.57±0.27 b	0.41±0.01 a	6.39±0.08 a	4.41±0.01 a	1.99±0.08 b
T3		41.00±3.06 g	36.67±1.20 d	7.51±0.01 c	0.40±0.01 a	3.49±0.02 d	2.94±0.15 b	0.55±0.16 e
T4		43.33±1.76 f	26.33±0.33 e	7.23±0.07 c	0.26±0.01 c	3.48±0.03 d	2.90±0.15 b	0.58±0.18 e
T5		46.67±1.67 ef	27.00±0.58 e	7.39±0.11 c	0.29±0.01 c	3.55±0.03 d	2.07±0.06 cd	1.48±0.05 cd
T6		49.00±2.08 e	31.00±0.58 e	7.67±0.03 c	0.31±0.01 b	3.79±0.02 c	2.21±0.24 c	1.58±0.22 c

Values presented as mean ± SE of 9 fish per each group with three replicates. Means in the columns with the same letter do not differ significantly ($P>0.05$). ALT: serum alanine aminotransferase, AST: aspartate aminotransferase.

Table 5. Changes in serum total protein, albumin, globulin, and A/G ratio in Nile tilapia (*O. niloticus*) injected with *P. aeruginosa*, streptomycin, and two phages after 3 and 12 days of infection

Parameters Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G
After 3 days				
T1	3.24±0.03 a	2.31±0.06 a	0.94±0.08 f	2.52±0.29 a
T2	2.33±0.03 h	1.28±0.04 e	1.06±0.04 ef	1.21±0.08 ef
T3	2.93±0.02 d	1.81±0.04 c	1.12±0.05 efg	1.63±0.11 cd
T4	3.09±0.05 bc	1.72±0.05 c	1.37±0.06 abc	1.26±0.08 cde
T5	3.07±0.04 bc	1.52±0.03 d	1.55±0.03 a	0.98±0.03 f
T6	3.04±0.04 c	2.09±0.07 b	0.95±0.05 f	2.23±0.18 ab
After 12 days				
T1	3.16±0.01 ab	1.86±0.03 c	1.30±0.04 bcd	1.44±0.07 cde
T2	2.48±0.04 g	1.43±0.02 de	1.05±0.05 ef	1.37±0.07 cde
T3	2.69±0.03 f	1.45±0.04 de	1.24±0.04 cde	1.17±0.07 ef
T4	2.90±0.00 de	1.78±0.13 c	1.12±0.13 efg	1.66±0.31 cd
T5	2.71±0.01 f	1.73±0.12 c	0.98±0.11 f	1.85±0.36 bc
T6	2.82±0.01 e	1.35±0.01 de	1.47±0.04 ab	0.92±0.05 f

Values presented as means ± SE of 9 fish per each group with three replicates. Means in the columns with the same letter do not differ significantly ($P>0.05$).

In this experiment, a cocktail of both phages (Ps1 and Ps2) was likely to inhibit bacterial growth for 80 h since there were fewer than 0.05 germs per cubic millimeter (OD_{600}). At the same time, the bacterial curve was 0.33 (OD_{600}) until the end of the experiment (80 h). Therefore, the optimal MOI of 0.01 was used in the following experiment (Figure 2). The combination of the AS-gs and AS-yj exhibited much more antibacterial activity than either phage alone, according to Chen et al. (2018). Kauppinen et al. (2021) observed that a cocktail of three phages showed a slightly more significant drop in the host level than individual phages.

Our results were comparable to those obtained by Hyman and Abedon (2010) and Castillo et al. (2015) who showed that these phages are promising for phage therapy. It is suggested that *in vitro* application of phages as a biocontrol agent against bacteria is a prerequisite experiment to determine which MOI should be chosen before the application in practice with fish in aquaculture. Because of the superiority of phages (Ps1 and Ps2) in the activation of bacterial growth, as shown in Figure 2, it could be proposed that both phages (Ps1 and Ps2) might have an essential role in *P. aeruginosa* growth control when both phages were a part of any cocktail.

The fish are vulnerable to *P. aeruginosa* with moderate to high losses; the premortal indications reported in our investigation are consistent with the findings of Som-siri and Soontornvit (2002) and Soror (2020). Fish infected with *Pseudomonas* spp., showed skin hemorrhages in the opercula, mouth, and ventral side of the body, according to Wakabayashi and Egusa (1972). *Pseudomonas* are harmful pathogen threatening fish that can lead to hemorrhagic septicemia and ulcerative syndrome, according

to Eissa et al. (2010). Several antibiotics are utilized to stop the spread of illnesses and foster fish development and health (Osselaer et al., 1997; Parker and Armstrong, 1987). Antibiotic misuse, however, can result in pathogenic resistance, damage the environment, and endanger the health of both humans and animals. Natural remedies like bacteriophages may be used to treat diseases, particularly in aquatic organisms like fish, especially for marine organisms like fish. This study's hematological alterations agree with Podeti and Benarjee (2017), who concluded that *Pseudomonas* infection reduced Hb and RBCs in freshwater mussel fish. The shallow values of Hb and RBCs may be indicative of anemia. Although the RBCs decreased, hematocrit (Hct%) was increased, possibly due to increased red cell volume. The increase in the MCV confirms this, which is termed macrocytic anemia.

The MCH and MCHC were decreased in fish infected by *P. aeruginosa* due to the hemolysis and inhibition of hemoglobin synthesis in the impaired liver in this fish group, as will be discussed later. Fish that received streptomycin or phages could overcome the bacterial infection, and these hematological parameters were improved, especially in fish injected with phage 2, followed by the other agents. RDW is a good indicator of the degree of anisocytosis. The life of RBC is lowered in the presence of oxidative stress, which reveals an elevation in hemolysis and then an increase in RDW (Li et al., 2017). The results indicate a more considerable RDW value in group T2 than in the control group T1. RDW can be considered an inflammatory reaction indicator (Wang et al., 2014). Elevated RDW can be present because of the elevation in oxidative stress on RBC that leads to

damaged cells and destruction in the tissue (Kurtoğlu et al., 2013).

Platelets help leukocytes travel from the bloodstream to the tissues by facilitating their passage through the channel wall. Platelets can produce reactive oxygen species, or oxidative stress, associated with inflammatory reactions. They can also play a significant role in disease pathogenesis. It is well known that phages can treat diseases brought on by bacteria that are multidrug resistant. Due to selectivity, using a single phage type to treat bacterial infections is rarely necessary (Castillo et al., 2015; Yen et al., 2017).

It was noticed that the platelets count (Plt) and platelet crit (PCT) were low. In contrast to elevated MPV and PDW, this confirmed that platelet numbers are positively correlated with PCT but negatively correlated with MPV and PDW, as previously mentioned by Yilmaz and Yilmaz (2016) during early endotoxemia in dogs. In humans, Nelson and Kehl (1981) noticed that in acute infection, there was a platelet decrease associated with an elevation in MPV.

During inflammation, platelets interact with leukocytes, forming platelet-leukocyte aggregates via adhesion proteins expressed on the cell surface during activation. In addition, platelets support leukocytes to overcome bacterial infections by direct contact, encapsulation of bacteria, and production of reactive oxygen species and platelet microbicidal proteins. Osselaer et al. (1997) reported that PDW indicates volume variability in platelet size and is elevated in the presence of platelet anisocytosis. Vagdatli et al. (2010) found a direct relationship between MPV and PDW, and both are usually altered in the same way under certain physiological conditions. Other studies showed a conflict in the relationship between platelet volume and count. According to these findings, platelet indices are influenced in various ways (Chandrashekar, 2013; Mariani et al., 2014; Yang et al., 2006).

Elevated MPV indicates elevated platelet diameter, a marker of production rate and platelet activation. During activation, platelets' shapes alter from biconcave discs to spherical, and a pronounced pseudopod formation leads to MPV elevation during platelet activation. Becchi et al. (2006) found that MPV was critical to predicting the problem at an early phase of sepsis.

Changes in total counts of the white blood cells (WBCs) and their differential types indicate a stress condition in *O. niloticus* due to *P. aeruginosa*. Previous studies confirmed that, under heavy infection, the organism releases more white blood cells, and lymphocytes have been reported as competent immune cells. Infection stress increases neutrophils. Fish neutrophils are the source of lysozyme, which damages the cell walls of bacteria, leading to their damage (Ellis, 2001; Kumar et al., 2013). Also, Ellis (1999) and Basha et al. (2013) confirmed the critical role of lysozyme in innate immunity by lysis of bacterial cell walls, thus enhancing the phagocytosis of bacteria.

These findings were confirmed by Xia et al. (2017). The increases in WBCs in the phage-injected groups (T4, T5, and T6) were lower than those in fish injected with *P. aeruginosa*, which may be because of the lowering of bacterial stress. The disturbances in the ALT, AST, urea, creatinine, and bilirubin levels indicate liver and kidney dysfunction due to infection stress by *P. aeruginosa*. Magdy et al. (2014) and Soror (2020) recorded the same results in African catfish and common carp infected by *P. aeruginosa*. The increase in the ALT and AST on the third day and 12 days post-infection might be due to the liver's damage to the enzymes' storage cells, releasing the stored enzymes into the bloodstream. The synthesizing cells were damaged, and the enzyme synthesis was inhibited, leading to a decrease in their activities in the bloodstream and the fish then became exhausted and died. Biochemical analysis has significance in examining altered enzyme activity produced by a foreign toxicant. A rapid increase in ALT and AST activity is a chief indicator of hepatic dysfunction. Pathogenic infections elevate the release of liver enzymes by hindering their normal activation, as the liver exports these enzymes into the bloodstream in fish (Pérez-Rostro et al., 2004). Current findings reveal that liver enzyme activities significantly differed between the infected and the control groups. Increased ALT, AST, and ALP enzyme activity in *L. rohita* infected with *A. hydrophila* was previously reported (Manoj et al., 2010). Significant serum ALT, AST, and ALP variations were reported after fungal (*C. albicans*) infection in *O. niloticus* (Zayed et al., 2016).

Urea and creatinine are increased in fish infected by *P. aeruginosa* due to kidney failure; however, the fish recovered in Ps1 and Ps1+Ps2 treated fish. The fish overcame the infection and resisted the stress in the other groups treated with streptomycin or phages; our results agree with Amer et al. (2019).

Bilirubin is made from the destruction of aged RBCs. The amount of bilirubin in the blood should be small. Some bilirubin is bound to the albumin in the blood. This kind of bilirubin is known as unconjugated or indirect bilirubin. In this study, total and direct bilirubin increased significantly ($P < 0.05$) in fish infected by *P. aeruginosa* due to the continuous hemolysis of RBCs and Hb as well as liver dysfunction as described by Chinabut (2002) and Abbas et al. (2016).

Indirect bilirubin is unconjugated, and the critical part of bilirubin is formed in the bile. Herein, indirect bilirubin decreased due to the impaired liver because of *P. aeruginosa* infection, while direct bilirubin increased and was then excreted. On the other hand, indirect bilirubin showed the inverse direction. In fish groups treated with streptomycin or phages, all the biochemical changes were improved compared to those in fish infected by *P. aeruginosa*, but still lower than the control.

Reduction in total protein and albumin confirms the liver cell damage and impairment of protein synthesis in the liver. In addition, it may result from malnutrition due to loss of appetite due to bacterial infection. Also, the re-

duction in protein levels in the blood might be due to the dysfunction of the kidneys, which pass the proteins outside. Bacterial load reduction enhanced the protein profile in fish groups treated with antibiotics or phages. Globulins were increased to form the antibodies against infection, and consequently, the A/G ratio increased.

Conclusion

It could be concluded that phages help control *P. aeruginosa* infection and improve hematological and biochemical measures in Nile tilapia. Using phages instead of antibiotics is recommended to protect the aquatic environment against antibiotic-resistant bacterial strains and consequently protect ourselves against zoonotic diseases. More studies on *in vivo* methods are needed to estimate the potency of the phage cocktail (Ps1 and Ps2) for the biocontrol of *P. aeruginosa* diseases in aquaculture.

Acknowledgment

The authors gratefully acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R31), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through large group Research Project under grant number RGP 2/12/44.

Conflict of interest

The authors declare no conflicts of interest.

References

- Abbas M.W., Shamshad T., Ashraf M.A., Javaid R. (2016). Jaundice: a basic review. *Int. J. Res. Med. Sci.*, 4: 1313–1319.
- Abd El-Hack M.E., El-Saadony M.T., Nader M.M., Salem H.M., El-Tahan A.M., Soliman S.M., Khafaga A.F. (2022 a). Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). *Int. J. Biometeorol.*, 66: 2183–2194.
- Abd El-Hack M.E., El-Saadony M.T., Elbestawy A.R., Nahed A., Saad A.M., Salem H.M., El-Tahan A.M., Khafaga A.F., Taha A.E., AbuQamar S.F., El-Tarabily K.A. (2022 b). Necrotic enteritis in broiler chickens: disease characteristics and prevention using organic antibiotic alternatives – a comprehensive review. *Poultry Sci.*, 101: 101590.
- Abedon S.T., Thomas-Abedon C. (2010). Phage therapy pharmacology. *Curr. Pharm. Biotechnol.*, 11: 28–47.
- Adesoji A.T., Abubakar F., Ipinlaye J.S. (2016). Occurrence of antibiotic resistant bacteria in faeces from abattoir waste, processing water and products from Dutsin-Ma, Katsina State, Nigeria. *J. Bacteriol. Mycol.*, 3: 1022.
- Amer S.A., Osman A., Al-Gabri N.A., Elsayed S.A.M., Abd El-Rahman G.I., Elabbasy M.T., Ahmed S.A.A., Ibrahim R.E. (2019). The effect of dietary replacement of fish meal with whey protein concentrate on the growth performance, fish health, and immune status of Nile tilapia fingerlings, *Oreochromis niloticus*. *Animals*, 9: 1003.
- Arduro A., Linde A.R., Garcia-Vazquez E. (2013). Genetic detection of *Pseudomonas* spp. in commercial Amazonian fish. *Int. J. Environ. Res. Public Health*, 10: 3954–3966.
- Basha K.A., Raman R.P., Prasad K.P., Kumar K., Nilavan E., Kumar S. (2013). Effect of dietary supplemented andrographolide on growth, non-specific immune parameters and resistance against *Aeromonas hydrophila* in *Labeo rohita* (Hamilton). *Fish Shellfish Immunol.*, 35: 1433–1441.
- Becchi C., Al Malyan M., Fabbri L., Marsili M., Boddi V., Boncinelli S. (2006). Mean platelet volume trend in sepsis: is it a useful parameter? *Minerva Anesthesiol.*, 72: 749–756.
- Blaxhall P., Daisley K. (1973). Routine haematological methods for use with fish blood. *J. Fish Biol.*, 5: 771–781.
- Castillo D., Christiansen R.H., Dalsgaard I., Madsen L., Middelboe M. (2015). Bacteriophage resistance mechanisms in the fish pathogen *Flavobacterium psychrophilum*: linking genomic mutations to changes in bacterial virulence factors. *Appl. Environ. Microbiol.*, 81: 1157–1167.
- Catho G., Martischang R., Boroli F., Chraïti M., Martin Y., Koyluk Tomsuk Z., Renzi G., Schrenzel J., Pugin J., Nordmann P. (2021). Outbreak of *Pseudomonas aeruginosa* producing VIM carbapenemase in an intensive care unit and its termination by implementation of waterless patient care. *Critical Care*, 25: 1–10.
- Chandrashekar V. (2013). Plateletcrit as a screening tool for detection of platelet quantitative disorders. *J. Hematol.*, 2: 22–26.
- Chen H., Cheng J., Ran L., Yu K., Lu B., Lan G., Lu F. (2018). An injectable self-healing hydrogel with adhesive and antibacterial properties effectively promotes wound healing. *Carbohydr. Polym.*, 201: 522–531.
- Chinabut S. (2002). Jaundice disease in catfish, a case study demonstrating a decline in incidence as a result of research output. In: *Primary Aquatic Animal Health Care in Rural, Small-scale, Aquaculture Development*, J.R. Arthur, M.J. Phillips, R.P. Subasinghe, M.B. Reantaso, I.H. MacRae (eds.). FAO Fish. Tech. Pap., 406: 77–80.
- Clinical and Laboratory Standard Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement. In: Wayne, PA 2021; M100-S25.
- Crone S., Vives-Flórez M., Kvich L., Saunders A.M., Malone M., Nicolaisen M.H., Martínez-García E., Rojas-Acosta C., Catalina Gomez-Puerto M., Calum H. (2020). The environmental occurrence of *Pseudomonas aeruginosa*. *Apmis*, 128: 220–231.
- Dacie S.J.V., Lewis S.M. (1984) *Practical haematology*. 6th Edition, Churchill Livingstone, Edinburgh, London, pp. 22–27.
- Donati V.L., Dalsgaard I., Sundell K., Castillo D., Er-Rafik M., Clark J., Wiklund T., Middelboe M., Madsen L. (2021). Phage-mediated control of *Flavobacterium psychrophilum* in aquaculture: *In vivo* experiments to compare delivery methods. *Front. Microbiol.*, 12: 628309.
- Doss J., Culbertson K., Hahn D., Camacho J., Barekzi N. (2017). A review of phage therapy against bacterial pathogens of aquatic and terrestrial organisms. *Viruses*, 9: 50.
- Eissa N., El-Ghiet E., Shaheen A., Abbas A. (2010). Characterization of *Pseudomonas* species isolated from tilapia "*Oreochromis niloticus*" in Qaroun and Wadi-El-Rayan lakes, Egypt. *Glob. Vet.*, 5: 116–121.
- El-Houseiny W., Mansour M.F., Mohamed W.A.M., Al-Gabri N.A., El-Sayed A.A., Altohamy D.E., Ibrahim R.E. (2021). Silver nanoparticles mitigate *Aeromonas hydrophila*-induced immune suppression, oxidative stress, and apoptotic and genotoxic effects in *Oreochromis niloticus*. *Aquaculture*, 535: 736430.
- El-Saadony M.T., Alagawany M., Patra A.K., Kar I., Tiwari R., Dawood M.A., Dhama K., Abdel-Latif H.M.R. (2021). The functionality of probiotics in aquaculture: An overview. *Fish Shellfish Immunol.* 117: 36–52.
- El-Saadony M.T., Swelum A.A., Ghanima M.M.A., Shukry M., Omar A.A., Taha A.E., Salem H.M., El-Tahan A.M., El-Tarabily K.A., Abd El-Hack M.E. (2022 a). Shrimp production, the most important diseases that threaten it, and the role of probiotics in confronting these diseases: A review. *Res. Vet. Sci.*, 144: 126–140.
- El-Saadony M.T., Salem H.M., El-Tahan A.M., Abd El-Mageed T.A., Soliman S.M., Khafaga A.F., Swelum A.A., Ahmed A.E., Alshammari F.A., Abd El-Hack M. E. (2022 b). The control of poultry salmonellosis using organic agents: an updated overview. *Poultry Sci.*, 101: 101716.
- El Zlitne A. (2022). Heavy metals concentration patterns in the At-

- lantic horse meckrel (*Trachurus trachurus*), the round sardinella (*Sardinella aurita*) and the common panadora (*Pagellus erythrinus*) from northwestern Egyptian coasts. Egypt. J. Aquat. Biol. Fish., 26: 63–81.
- Ellis A. (1999). Immunity to bacteria in fish. Fish Shellfish Immunol., 9: 291–308.
- Ellis A. (2001). Innate host defense mechanisms of fish against viruses and bacteria. Dev. Comp. Immunol., 25: 827–839.
- Elsayed M., Essawy M., Shabana I., Abou El-Atta M., EL-Banna N. (2018). Studies on bacterial pathogens in some marine fishes in EL-Mansoura, Egypt. Amer. J. Agric. Biol. Sci., 13: 9–15.
- Golkar Z., Bagasra O., Pace D.G. (2014). Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. J. Infect. Dev. Ctries., 8: 129–136.
- Henry R.J. (1964). Clinical chemistry, principles and techniques. Hoeber Medical, Harper-Row, 190: 287.
- Hodgson K. (2013). Bacteriophage therapy. Microbiol. Aust., 34: 28–31.
- Housby J.N., Mann N.H. (2009). Phage therapy. Drug Discov. Today, 14: 536–540.
- Hyman P., Abedon S.T. (2010). Bacteriophage host range and bacterial resistance. Adv. Appl. Microbiol., 70: 217–248.
- Iliev I., Marhova M., Gochev V., Tsankova M., Trifonova S. (2015). Antibiotic resistance of Gram-negative benthic bacteria isolated from the sediments of Kardzhali Dam (Bulgaria). Biotechnol. Biotechnol. Equip., 29: 274–280.
- Jamalzadeh H., Keyvan A., Ghomi M., Gherardi F. (2009). Comparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*). Afr. J. Biotechnol., 8: 319–322.
- Kaplan A., Glucose A. (1984). Clinical chemistry. The CV Mosby Co., St Louis, Toronto, Princeton, 436 pp.
- Kauppinen A., Siponen S., Pitkänen T., Holmfeldt K., Pursiainen A., Torvinen E., Miettinen I.T. (2021). Phage biocontrol of *Pseudomonas aeruginosa* in water. Viruses, 13: 928.
- Kelly D., McAuliffe O., O'Mahony J., Coffey A. (2011). Development of a broad-host-range phage cocktail for biocontrol. Bioeng. Bugs, 2: 31–37.
- Kumar S., Raman R., Pandey P., Mohanty S., Kumar A., Kumar K. (2013). Effect of orally administered azadirachtin on non-specific immune parameters of goldfish *Carassius auratus* (Linn. 1758) and resistance against *Aeromonas hydrophila*. Fish Shellfish Immunol., 34: 564–573.
- Kurtoglu E., Aktürk E., Korkmaz H., Sincer İ., Yılmaz M., Erdem K., Celik A., Ozdemir R. (2013). Elevated red blood cell distribution width in healthy smokers. Turk. Kardiyol. Dern. Ars., 41: 199–206.
- Lewis T.T., Everson-Rose S.A., Powell L.H., Matthews K.A., Brown C., Karavolos K., Wesley D. (2006). Chronic exposure to everyday discrimination and coronary artery calcification in African-American women: the SWAN Heart Study. Psychosom. Med., 68: 362–368.
- Li W.H., Song Y.C., Zhang H., Zhou Z.J., Xie X., Zeng Q.N., Guo K., Wang T., Xia P., Chang D.M. (2017). Decreased expression of Hsa_circ_00001649 in gastric cancer and its clinical significance. Dis. Markers, 2017: 4587698.
- Lombarts A., Koevoet A., Leijnse B. (1986). Basic principles and problems of haemocytometry. Ann. Clin. Biochem., 23: 390–404.
- Ly-Chatain M.H. (2014). The factors affecting effectiveness of treatment in phages therapy. Front. Microbiol., 5: 51.
- Magdy I., El-Hady M., Ahmed H., Elmeadawy S., Kenwy A. (2014). A contribution on *Pseudomonas aeruginosa* infection in African catfish (*Clarias gariepinus*). Res. J. Pharm. Biol. Chem. Sci., 5: 575–588.
- Manna S., Bera A., Baitha R., Das N. (2023). Fish diseases and their management in inland open waters (Chapter). In: Advances in fish research: inland aquatic ecosystem health and fisheries. Narendra Publishing House, Delhi, India.
- Manoj C., Nair C.M., Patel M., Salin K. (2010). Haematobiochemical and histopathological changes in *Labeo rohita* infected with *Aeromonas hydrophila* by immersion challenge. Fishery Technol., 47: 151–160.
- Mapes A.C., Trautner B.W., Liao K.S., Ramig R.F. (2016). Development of expanded host range phage active on biofilms of multi-drug resistant *Pseudomonas aeruginosa*. Bacteriophage, 6: e1096995.
- Mariani E., Filardo G., Canella V., Berlingeri A., Bielli A., Cattini L., Landini M.P., Kon E., Marcacci M., Facchini A. (2014). Platelet-rich plasma affects bacterial growth *in vitro*. Cytotherapy, 16: 1294–1304.
- McKnight I.M. (1966). A hematological study on the mountain whitefish, *Prosopium williamsoni*. J. Fish. Res., 23: 45–64.
- Nelson R.B. 3rd, Kehl D. (1981). Electronically determined platelet indices in thrombocytopenic patients. Cancer, 48: 954–956.
- Osselaer J.-C., Jamart J., Scheiff J.-M. (1997). Platelet distribution width for differential diagnosis of thrombocytosis. Clin. Chem., 43: 1072–1076.
- Parker D., Armstrong D. (1987). Antibiotic feed additives and live-stock production. Proc. Nutr. Soc., 46: 415–421.
- Patton C., Crouch S. (1977). Enzymatic colorimetric method to determine urea in serum. Anal. Chem., 49: 464–469.
- Pereira C., Silva Y.J., Santos A.L., Cunha Â., Gomes N.C., Almeida A. (2011). Bacteriophages with potential for inactivation of fish pathogenic bacteria: survival, host specificity and effect on bacterial community structure. Mar. Drugs, 9: 2236–2255.
- Pérez-Rostro C.I., Racotta I.S., Ibarra A.M. (2004). Decreased genetic variation in metabolic variables of *Litopenaeus vannamei* shrimp after exposure to acute hypoxia. J. Exp. Mar. Biol. Ecol., 302: 189–200.
- Petrie A., Watson P. (2013). Statistics for veterinary and animal science. John Wiley & Sons, 480 pp.
- Podeti K.R., Benarjee G. (2017). Haematological changes in South Indian fresh water murrel, *Channa punctatus* have both EUS and *A. hydrophila* infection. J. Parasit. Dis., 41: 329–335.
- Řehulka J. (2000). Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 190: 27–47.
- Reitmann S. (1957). Colorimetric method for the determination of serum glutamic pyruvate and glutamic oxaloacetate transaminase. Amer. J. Clin. Path., 28: 56–63.
- Saad A.M., Sitohy M.Z., Sultan-Alolama M.I., El-Tarabily K.A., El-Saadony M.T. (2022). Green nanotechnology for controlling bacterial load and heavy metal accumulation in Nile tilapia fish using biological selenium nanoparticles biosynthesized by *Bacillus subtilis* AS12. Front. Microbiol., 13: 1015613.
- Somsiri T., Soontornvit S. (2002). Bacterial diseases of cultured tiger frog (*Rana tigrina*). Diseases in Asian aquaculture IV. Proc. Fourth Symposium on Diseases in Asian Aquaculture, 22–26.11.1999, Cebu City, Philippines. Fish Health Section, Asian Fisheries Society, pp. 427–434.
- Soror E.M. (2020). Effect of flavomycin and synbiotic on experimentally-infected common carp with *Pseudomonas aeruginosa*. Benha Vet. Med. J., 39: 189–193.
- Summarwar S., Verma S. (2012). Study of selected haematological indices of freshwater fish from Bisalpur reservoir. Int. J. Fundament Appl. Lif. Sci., 2: 51–54.
- Swelum A.A., Elbestawy A.R., El-Saadony M.T., Hussein E.O., Alhotan R., Suliman G.M., Taha A.E., Ba-Awad H., El-Tarabily K.A., Abd El-Hack M. E. (2021). Ways to minimize bacterial infections, with special reference to *Escherichia coli*, to cope with the first-week mortality in chicks: an updated overview. Poultry Sci., 100: 101039.
- Vagdatli E., Gounari E., Lazaridou E., Katsibourlia E., Tsikopoulou F., Labrianou I. (2010). Platelet distribution width: a simple, practical and specific marker of activation of coagulation. Hippokratia, 14: 28–32.
- Wakabayashi H., Egusa S. (1972). Characteristics of a *Pseudomonas* sp. from an epizootic of pond-cultured eels (*Anguilla japonica*). Bull. Jpn. Soc. Sci. Fish., 38: 577–587.
- Wang F.-M., Xu G., Zhang Y., Ma L.-L. (2014). Red cell distribution width is associated with presence, stage, and grade in patients with renal cell carcinoma. Dis. Markers, 2014: 860419.
- Wotton I.D.H.F. (1982). Microanalysis in medical biochemistry. Churchill, New York, USA.
- Wright A., Hawkins C., Ånggård E., Harper D. (2009). A controlled

- clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clin. Otolaryngol., 34: 349–357.
- Xia H., Tang Y., Lu F., Luo Y., Yang P., Wang W., Jiang J., Li N., Han Q., Liu F. (2017). The effect of *Aeromonas hydrophila* infection on the non-specific immunity of blunt snout bream (*Megalobrama amblycephala*). Cent. Eur. J. Immunol., 42: 239–243.
- Yang A., Pizzulli L., Lüderitz B. (2006). Mean platelet volume as marker of restenosis after percutaneous transluminal coronary angioplasty in patients with stable and unstable angina pectoris. Thromb. Res., 117: 371–377.
- Yilmaz T., Yilmaz A. (2016). Relationship between altered platelet morphological parameters and retinopathy in patients with type 2 diabetes mellitus. J. Ophthalmol., 2016: 9213623.
- Zayed M., Soliman M., Kalil R., Saad T. (2016). Isolation of *Candida albicans* from naturally infected freshwater fish. Kafrelsheikh Vet. Med. J., 14: 21–45.
- Zhang H., Yang Z., Zhou Y., Bao H., Wang R., Li T., Pang M., Sun L., Zhou X. (2018). Application of a phage in decontaminating *Vibrio parahaemolyticus* in oysters. Int. J. Food Microbiol., 275: 24–31.

Received: 17 VI 2023

Accepted: 26 X 2023