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Bacteriophage Action against Tilapia Fish *Aeromonasis* in Aquarium Tanks in Uganda

TAMALE, A.[1]*, LABALPINY, L. D.[1], SSENFUMA, R.[1], WALAKIRA, J. K.[3], WAMALA, S. P.[2], KAMYA, D. [2], ALAFI, S.[2], LWESYA, R. H.[2], MUGASA, C. M.[4], MAYANJA, M. N.[5], EJOBI, F.[5] and NAKAVUMA, J.[2]

[1]Department of Wildlife and Aquatic Animal Resources. School of Animal Resources and Veterinary Medicine. College of Veterinary Medicine, Animal Resources and Biosecurity. Makerere University, P.O. Box 7062, Kampala, Uganda
[2]Department of Bimolecular Resources & Biolab Sciences. (BBS). School of Biosecurity, Biotechnical & Lab. Sciences. College of Veterinary Medicine, Animal Resources and Biosecurity. Makerere University, P.O. Box 7062, Kampala, Uganda
[3]National Fishery Resources Research Institute- NAFIRRI, Uganda. P.O. Box 343 Jinja, Uganda
[4]Department of Biotechnical & Diagnostic Sciences. (BDS). School of Biosecurity, Biotechnical & Lab. Sciences. College of Veterinary Medicine, Animal Resources and Biosecurity. Makerere University, P. O. Box 7062, Kampala, Uganda
[5]Department of Biosecurity, Ecosystems & Vet Public Health. (BEP). School of Biosecurity, Biotechnical & Lab. Sciences. College of Veterinary Medicine, Animal Resources and Biosecurity. Makerere University, P.O. Box 7062, Kampala, Uganda
Corresponding Author: andietam@gmail.com

ABSTRACT

Aeromonas is one of the most virulent fish pathogens known in the universe. *Aeromonas hydrophila* causes a fish disease known as "Aeromonas or Hemorrhagic Septicemia or Motile Aeromonas Septicemia or Ulcer Disease or Red-Sore Disease". Management involves both prophylactic and therapeutic use of antibiotics. Unfortunately, the indiscriminate use of antimicrobial agents has led to the development of resistant strains of bacteria. The need for alternative strategies other than antibiotics has stimulated research into lytic bacteriophages because of the lower chance of bacteria developing resistance. This study assessed the effect of using bacteriophages on *Aeromonas hydrophila* in the treatment of tilapia fish Aeromonasis. The host was resuscitated and reconfirmed using biochemical tests and genetic profiling. The profiled bacteriophage was bulked up and enumerated using the overlay agar method. Bacteriophage stability in tank water for pH and temperature levels was established. Fish fingerlings of an average weight of 5 gm were acclimatized, put in aquarium glass water tanks and separated into test and control subjects. Test subjects were divided into two groups, one inoculated with bacteria, stressed through delayed feeding and bacteriophages added to the tanks. While the second test group had bacteriophage added without stress. The control groups were not inoculated with either bacteria or phages. Bacteriophages exhibited stability at both pH 6-9 in tank water. The bathing method was found to be effective for fish challenge and phage application. Stress influenced fish disease onset in the tanks. A multiplicity of infection of 0.01, 0.1, and 1 showed efficacy on clearing with no differences, while 10 and 100 had outstanding results. Bacteriophage dissociation took around 14 days from the treatment day. The survival curves plotted using GraphPad Prism version 5 using the Log-rank (Mantel-Cox) test revealed a significant difference in the survival of fish (Chi square = 20.92, df =2 and P value <0.0001 in both test and control groups. The log-rank test for trends didn't reveal a significant difference (Chi square = 1.934, df = 1 and P value = 0.1643). The non-parametric test for

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differences in the survival curves using the Gehan-Breslow-Wilcoxon test revealed a significant difference between the curves (Chi square = 19.67, df =2 and P value <0.0001). Use of a single phage isolate showed control of Aeromonas hydrophila infection in water tanks. Direct administration of phages to tank water is an effective route for both phage application and bacterial challenge. Future studies were recommended to establish phage stability in pond water and the need to test the survival of fish in pond water infested with *Aeromonas hydrophila* using cocktail phage prepared from the three profiled isolates.

Keywords: Bacteriophage, Aquaculture, Aeromonas hydrophila, Fish treatment, Aeromoniasis

RÉSUMÉ

Aeromonas est l'un des agents pathogènes les plus virulents des poissons connus. Aeromonas hydrophila provoque une maladie des poissons connue sous les noms de « maladie à Aeromonas », « septicémie hémorragique », « septicémie à Aeromonas mobile », « maladie des ulcères » ou « maladie des plaies rouges ». La gestion de cette maladie implique à la fois des mesures prophylactiques et thérapeutiques utilisant des antibiotiques. Malheureusement, l'utilisation indiscriminée des agents antimicrobiens a conduit au développement de souches bactériennes résistantes. Le besoin de stratégies alternatives autres que les antibiotiques a stimulé la recherche sur les bactériophages lytiques, en raison du faible risque de développement de résistance bactérienne. Cette étude a évalué l'effet de l'utilisation de bactériophages contre Aeromonas hydrophila dans le traitement de l'Aeromonose chez le tilapia. L'hôte a été réactivé et reconfirmé à l'aide de tests biochimiques et de profilage génétique. Le bactériophage profilé a été multiplié et dénombré par la méthode de surcouche d'agar. La stabilité du bactériophage dans l'eau des aquariums a été évaluée en fonction du pH et de la température. Des alevins de poisson d'un poids moyen de 5 g ont été acclimatés, placés dans des aquariums en verre et séparés en groupes test et témoin. Les sujets test ont été divisés en deux groupes : l'un inoculé avec la bactérie, stressé par un retard d'alimentation et traité avec les bactériophages ; le second groupe test a reçu les bactériophages sans stress. Les groupes témoins n'ont été inoculés ni avec les bactéries ni avec les phages. Les bactériophages ont montré une stabilité dans l'eau à un pH compris entre 6 et 9. La méthode de bain s'est révélée efficace pour le défi infectieux et l'application des phages. Le stress a influencé l'apparition de la maladie chez les poissons dans les aquariums. Une multiplicité d'infection de 0,01, 0,1 et 1 a montré une efficacité d'élimination sans différences notables, tandis que les multiplicateurs 10 et 100 ont donné des résultats exceptionnels. La dissociation des bactériophages a pris environ 14 jours après traitement. Les courbes de survie, tracées avec GraphPad Prism version 5 et le test Log-rank (Mantel-Cox), ont révélé une différence significative dans la survie des poissons (Chi carré = 20,92, ddl = 2 et valeur p < 0,0001) entre les groupes test et témoin. Le test Log-rank pour les tendances n'a pas montré de différence significative (Chi carré = 1,934, ddl = 1 et valeur p = 0,1643). Le test non paramétrique de Gehan-Breslow-Wilcoxon a révélé une différence significative entre les courbes (Chi carré = 19,67, ddl = 2 et valeur p < 0,0001). L'utilisation d'un seul isolat de phage a permis de contrôler l'infection à Aeromonas hydrophila dans les aquariums. L'administration directe de phages dans l'eau des aquariums est une méthode efficace pour l'application des phages et le défi bactérien. Des études futures sont recommandées pour établir la stabilité des phages dans les étangs et tester la survie des poissons dans de telles eaux contaminées par Aeromonas hydrophila, en utilisant un cocktail de phages préparé à partir des trois isolats profilés.

Mots clés: Bactériophage, Aquaculture, Aeromonas hydrophila, Traitement des poissons, Aeromonose

INTRODUCTION

Aeromonas bacteria are responsible for mortalities in fish hatcheries worldwide, hence the high prevalence of Aeromoniasis (Dang *et al*., 2021) . Due to the high prevalence, most fish hatcheries report hemorrhagic septicemia, and this is mostly attributed to *Aeromonas hydrophila.* These losses render it impossible for

the average Ugandan to attain the FAO requirement of 20.5 kg of fish per year (Maldonado-Miranda *et al*., 2022). Fish hatchery farmers have therefore attempted to use antibiotics to control Aeromonasis. The injudicious use of antibiotics has resulted in antibiotic resistance, a challenge that has no quick fix. Antimicrobial resistance is a threat to food security due to its adverse effects on One Health, a paradigm which looks at human, animal and environmental health (Kiggundu *et al*., 2024).

Due to the challenge of antibiotic resistance, alternatives to antibiotic use, such as bacteriophages, have been implemented (Żaczek *et al*., 2020). Phage banks are seen as the practical solution to antibiotic resistance, especially in resource-limited settings like Uganda (Nagel *et al*., 2022). This phage therapy will not work in isolation since surveillance of fish kills and reporting in real time is key in reducing overuse of antibiotics and large mortalities encountered especially in tilapia hatcheries (Isyagi-Levine *et al*., 2021.; Teplitz *et al*., 2025). A study by Deekshit *et al*. (2023) has demonstrated that Aeromonas organisms are some of the many bacteria which have shown antimicrobial resistance. Considering the "one health" approach of disease control, phage therapy looks to be the solution to multidrug resistance. This phage therapy is environmentally friendly, host-specific and self-limiting (Deekshit *et al*., 2023). The current study aimed at bulk production of a phage against *Aeromonas hydrophila* in pond and tanks. Phages

are viruses that affect bacteria and these lead to lysis of the bacteria which is

useful in prophylactic and therapeutic control. This destruction of bacteria also releases the nitrogen compounds from bacteria DNA back to the water system which is a favourable action for prokaryotes (Żaczek *et al*., 2020). Since phages are natural to the host environment where the fish live, it is prudent to get the phages from the water systems where the fish are cultured.

The phages produced from the water sources on farms in selected regions of the country were used to produce phages that will be utilised to control aeromonas in fish hatcheries. Since limited studies on phages have been conducted in sub-Saharan region of Africa, there is a need for more information to farmers about the alternatives to antibiotic use, creation of awareness about phage therapy and the source of phage production (Paquet *et al*., 2019; Dang *et al*., 2021). Phage therapy worldwide is accepted since it is environmentally friendly, specific for the organism targeted and self-regulating, (Dien *et al*., 2021). The choice of phage to use, i.e, monovalent or polyvalent, will depend on the isolates collected from the affected fish and the difference in genetic composition observed in the isolates. Whether a monovalent phage can work against similar bacteria or even cross protection is an aspect that the research team will watch keenly, since most studies recommend a cocktail for phage therapy (Nachimuthu *et al*., 2020). However, there is a need to demonstrate that the phages produced are lytic not lysogenic. The lysogenic phages are considered to have gene assortment, which might transfer resistance genes from one ecosystem to another, a predicament which the farmer is trying to avoid (JEPKURUI, 2023). This study is therefore establishing a single phage therapy against *A. hydrophila* in Aquarium tanks in Uganda.

MATERIALS AND METHODS

Study area. The study was executed at Makerere University, College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB). The College has a wet lab where fish trials are executed with other research teams and groups. The bacteria utilized in the study were isolated from fish farms all four regions of Uganda and these were archived in the Microbiology lab of the College, a certified lab in the country.

Bacteria isolation and confirmation. Archived bacterial isolates kept at the Veterinary Microbiology Laboratory at COVAB were accessed for the study. The bacteria were taken through the process of resuscitation using Trypticase Soy Broth (TSB), and this was incubated overnight at 30°C (Warburton *et al*., 1994). Pure cultures were obtained by sub-culturing on Trypticase Soy Agar (TSA) for colony morphology and on Blood agar (BA) to

demonstrate the hemolytic activity, and beta hemolysis was observed for *Aeromonas hydrophila* isolates. Biochemical analysis for purification of *Aeromonas hydrophila* isolates was achieved by biochemical tests, which included indole production, the reaction on Triple Sugar Iron (TSI) and Voges-Proskauer, citrate agar, and urease tests (IMVC-U) (Maalej *et al.,* 2004). Differential cultures were performed using Inositol where after 24 hrs incubation the white growth appeared. The isolate was molecular confirmed using 16 s genes as described by Kim *et al. (*2012) and Jun (2015).

Bulk production of phages. The bulk production of phages was designed as highlighted by (Luong, Salabarria, Edwards and Roach, n.d.).

Preparation of host bacteria for bulking: Cryotubes (cryovials) of *Aeromonas hydrophila* stock was taken from the deep freezer. Then 10 µl of the preserved bacteria was resuscitated in single strength TSB and incubated at 30°C overnight. This was the host bacteria for bulking up the bacteriophages,

Bulking up of the phages: Prior to administration phages were bulked up using the overlay method, 100 µl of an overnight culture of the *A. hydrophila* was added to 3 ml of overlay agar and then 100 ml of phage preparation was added in the same tube. The mixture was poured onto a TSA plate and allowed to solidify (approximately 5 minutes). The plate was incubated for 24 hours at 30°C. After incubation, 3 mls of SM buffer was poured onto a plate and put into an incubator shaker for 5-10 minutes to allow the phages to diffuse from the media. The SM buffer was transferred into a Falcon tube and filtered to remove remaining bacterial debris. The process was done several times until the required volume of phages was reached, and then quantification was followed using the serial dilution and overlay method (Anand *et al.,* 2016)

Phage stability at different physicochemical water parameters. Stability for acidic and alkaline conditions was assessed according to the methods described by (Dien *et al.,* 2021). The stability of the phages on different pH was tested where the phages were suspended in Phosphate buffer solution (PBS) adjusted with 1 M NaOH or HCl (Fisher Scientific, NJ), to yield a pH range of 6–9 (same pH range for fish ponds), and incubated at 30°C to determine their survival at 1, 2, 4, 6 and 8 hours. Equal bacteriophage concentrations (10^6) were subjected to different PH values ranging from 6-9, and the stability was assessed for 8 hours. Then the plaquing efficacy of the phage was retested as detailed by Tokman *et al.* (2016).

Direct release of phages in water. The method of phage release as described by (Anand *et al.,* 2020) involved a 20-litre tank on the test (phage+bacteria) where the MOI (Multiplicity of infection) of 1 was used. A second tank with the bacteria inoculated but not treated with phages acted as a positive control (bacteria only). The third tank without the bacteria acted as the negative control (nothing added). Each component was executed in triplicate to maintain the quality and statistical viability of results.

Bacteriophage dose determination. This was established using an MOI of 0.01, 0.1, 1, 10, and 100 as described by Kim *et al.* (2012) and Dien *et al.* (2021). The bacteria and phage were subjected to the same Falcon tube, and the concentration was adjusted to make a ratio of the aforementioned values. After 24 hours, the overlay method was used to determine the most effective bacteriophage dose using plaques on TSA. This experiment was done in replicates.

Bacteriophage efficiency in treating tilapia fingerlings. The bacteriophage efficiency was established as determined by Hoang (2018) and Le *et al.* (2018).The infected and control fingerlings were put in separate glass tanks of 20 L capacity containing water. Then 10 L of water, and 2ml of phage preparation were added to both tanks and containers. The bacteriophage estimations were performed daily using the overlay method while recording as well. The bacteriophage enumeration was perfomed on a daily basis while the mortalities were recorded as and when they occurred on a daily basis**.**

Study Design, data, and statistical analyses. An experimental study was employed to test the efficiency of bacteriophages in curing fish diseases. The study included the determination of the efficiency of phages on fingerlings and testing the bathing method as the route of administration. The bacteriophage efficiency data obtained from the laboratory experimentation for all methods tested were entered into Excel®2013, sorted and cleaned, and thereafter, exported to STATA® version 11 for analysing the resultant effects using two-way ANOVA and Survival analyses.

Quality control. Standard operating procedures from the veterinary microbiology research laboratory were utilized during the study. Before bulking up the phages, the plaquing assay was done to confirm the presence of the phage vials, and the host was reconfirmed. Bioinformatics sequencing of the bacteria was executed to ascertain purity. In the wet lab, experiments were done in triplicates, to reduce unexpected/uncontrollable deviations. High-grade reagents (AOAC approved) were used for analysis.

RESULTS

Bacteriophage stability in pH. Bacteriophage showed stability in all selected pH range with minimal variations in the concentrations of the bacteriophages. The bacteriophage concentration remained almost the same after 8 hours of the experiment, with just less than 1% reduction from the original concentration, as shown in Figure 1 below.

Infective dose determination. In the infective dosage experiment group, fish introduced to a bacterial load of 10^3 and above showed signs of disease within 48 hours, while a bacterial load of 10^2 and 10^1(CFU/ml) showed little to no signs which were not significant to the study. A higher concentration of 10^6 and 10^8 was more lethal to the subject even in some cases, succumbed the subjects to within 24-48 hours. The low bacterial dosage of 10^1 and 10^2 (CFU/ml) showed no effect to the subjects for all 5 days, while higher bacterial dosages above 10^2 showed dose-dependent mortalities. For instance, concentrations of 10^3 and 10^4(CFU/ml) showed percentage mortalities of 40% and 80% respectively, whereas the rest of the higher concentrations showed 100% mortalities.

Bacteriophage dosage determination. Efficacy of multiplicity of infection (MOI) of 0.01, 0.1, 1, 10 and 100 was assessed based on clearance on agar and optical density (OD) on broth. The MOIs of 0.01, 0.1, and 1 showed partial clearing with a trace of growth, while 10 and 100 showed complete clearance without any trace of growth. The dosage of 10 was selected as the best-fit minimum bactericidal concentration as shown in Table 1.

Survival of the fish. Finding indicates that the probability of survival was high among fish treated with phages (Tank2) compared to those not treated (Tank3) as shown figure 3. The survival probabilities for the phages treated group are higher than the survival probabilities for the untreated group, suggesting a survival benefit for the treated group. The Log-rank (Mantel-Cox) test showed a Chi square 20.92, df 2 and a P value <0.0001 which showed a difference between the survival curves

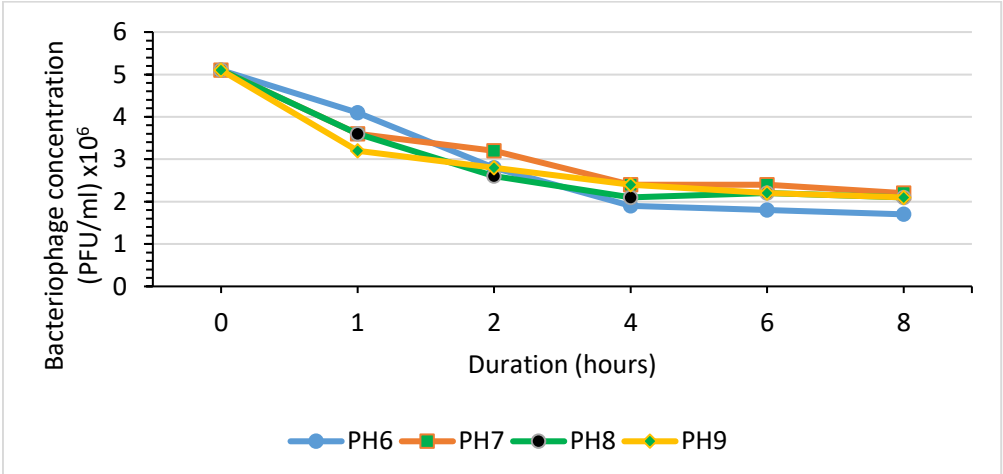

Table 1. Bacteriophage dosage determination

MOI	OD_{change}
0.01	0.164
0.1	0.11
1	0.13
10	0.07
100	0.07

The relationship between the MOI and OD shows a log (dose) vs. response curve with a LogEC50 of 1.787, standard error 1.908, 95% Confidence Intervals of -6.425 to 9.998 and R^2 value of 0.7779. The relationship between MOI and OD is shown in Figure 2 below and Kaplan-meter graph in Figure 3.

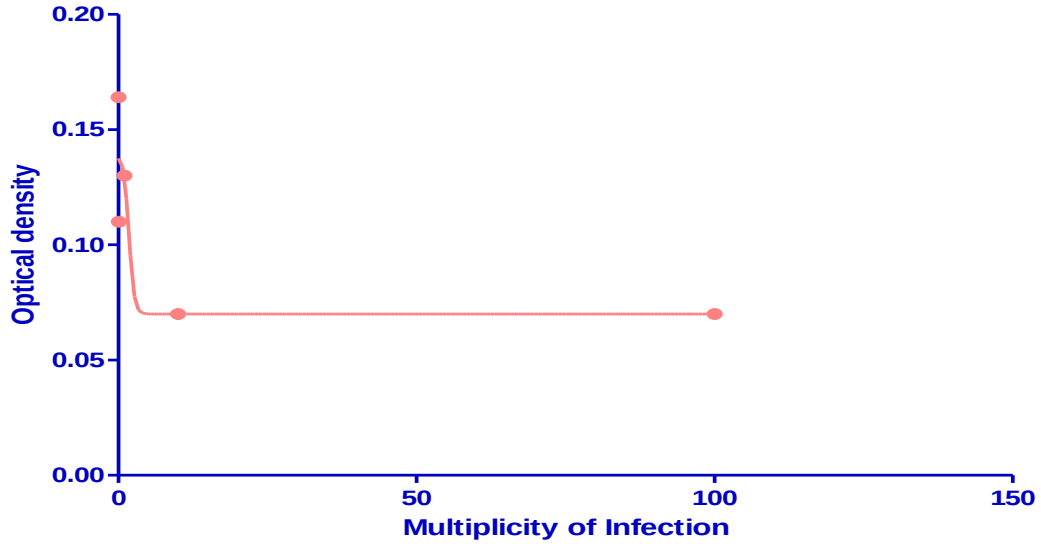

Figure 2. Relationship between Optical Density and Multiplicity of Infection

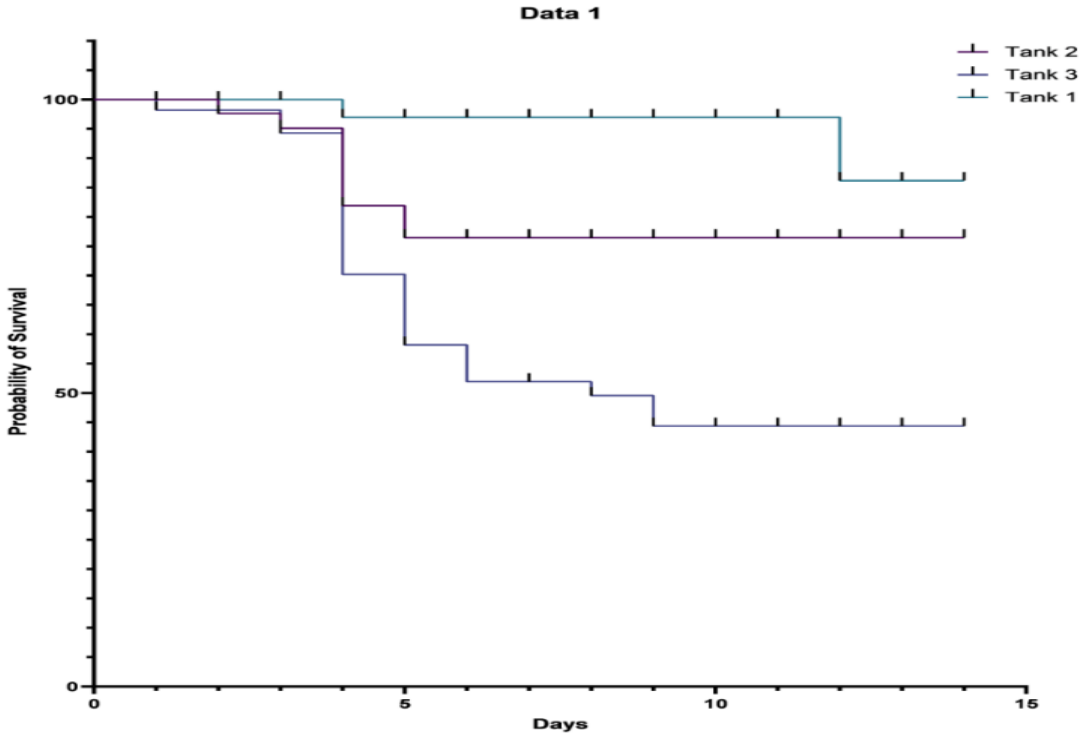

Figure 3. Kaplan–Meier graph of survival of fish in each

DISCUSSION

The study revealed that the optimal pH for bacteriophage action against *Aeromonas hydrophila* is within the range of 6 to 9. This establishment is crucial for bacteria control since most of the hatcheries have a similar pH range. The study also revealed that the bacteriophages remained consistent in number and efficiency at this pH range, with only 1% reduction in concentration after over 8 hours of action. Based on the fact that the phages are naturally obtained from the same ecosystem as the bacteria being controlled, there is minimal interference with the environment, and this is demonstrated by their ability to survive and maintain durability at various pH ranges. This is in agreement with Dien *et al*. (2021) who observed that bacteriophages were effective against a multidrug-resistant *Aeromonas hydrophila* species a pointer towards excellence when the phages are used in fish disease control. Temperatures are also crucial for phage plaquing and this was studied using phages against Listeria fish (Tokman *et al*., 2016).

Having observed the success of phage stability at indoor temperatures and pH, next in line was the evaluation of the doses which are suitable for action against the Aeromonas species studies.In the infective dose determination experiment, it was observed that higher bacterial loads, particularly at concentrations of 10^3 and 10^4 CFU/ml, resulted in higher mortality rates among the subjects. The fish mortality is influenced by extrinsic and intrinsic factors. Fish under sound management will be able to slow down the mortalities as the farmers seek, even at infection concentrations, such as those stated above (Chow and Rouf, 1983). Therefore, when challenging fish to establish the optimum dose, a large burden should not be put on the fish farm.

Fish exposed to bacterial loads of 10^3 CFU/ml showed a mortality rate of 40%, while those exposed to 10^4 CFU/ml exhibited a mortality rate of 80%. Conversely, lower bacterial doses of 10^1 and 10^2 CFU/ml did not significantly affect the fish during the 5-day study. These results suggest a dose-dependent relationship between bacterial load and mortality, emphasizing the importance of controlling bacterial populations to prevent adverse effects. These findings align with Schulz *et al.* (2019), who established similar percentages when working with eels.

The determination of bacteriophage dosage was assessed through the efficacy of multiplicity of infection (MOI) values ranging from 0.01 to 100. The results indicated that higher MOIs of 10 and 100 resulted in complete clearance of bacteria without any trace of growth. On the other hand, lower MOIs of 0.01, 0.1, and 1 only showed partial clearing with a trace of growth. The MOIs of the study were above those by Dien *et al.,* 2021) who observed MOIs of 0.1 and 1.0 showing survival percentages of about 50% and 73 % for Nile tilapia when challenged in combination with phages.

Based on these findings, a bacteriophage dosage of 10 was determined to be the best-fit minimum bactericidal concentration. This dosage exhibited effective bactericidal activity, eliminating the bacteria. The log EC50 demonstrates that phages are even in some instances better than antibiotics (Pereira *et al.,* 2021). The use of phages not only in Aquaculture has been demonstrated to be effective in other human-related foodborne illnesses (Easwaran *et al*., 2017)This will go a long way in ensuring that bacterial infections, especially those associated with drug resistance, are controlled or even cleared by phages at doses less than 10 MOI**.**

Specifically, the results presented in the study demonstrate that the stability of bacteriophages across a pH range of 6 to 9 has been confirmed. In addition, fish survival rates were examined in relation to bacteriophage treatment. Compared to untreated fish, those treated with bacteriophages had a higher likelihood of survival. There was a consistent increase in survival probabilities for the treated group, indicating a positive effect of bacteriophage treatment on the survival of the fingerlings. The statistical analysis, specifically the Log-rank (Mantel-Cox) test, further supported the differences in survival between the treated and untreated groups. The Chi-square value of 20.92, with 2 degrees of freedom and a p-value less than 0.0001, indicated a significant

difference between the survival curves of the two groups. This analysis strengthens the conclusion that bacteriophage treatment positively impacted the survival of the fish subjects.

CONCLUSION

The study provides valuable insights into bacteriophage stability across pH ranges, bacterial infectivity levels, determination of bacteriophage dosage, and survival benefits associated with bacterial phage treatment.

ETHICAL CONSIDERATIONS

Ethical clearance was obtained from the Institutional Review Board of Makerere University College of Veterinary Medicine, Animal Resources, and Biosecurity (COVAB) and the Uganda National Council of Science and Technology.

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REFERENCES

Anand, T., Vaid, R. K., Bera, B. C., Singh, J., Barua, S., Virmani, N., K., R., Yadav, N. K., Nagar, D., Singh, R. K. and Tripathi, B. N. 2016. Isolation of a lytic bacteriophage against *virulent Aeromonas hydrophila* from an organized equine farm. *Journal of Basic Microbiology*. https://doi.org/10.1002/jobm.201500318

Anand, T., Virmani, N., Kumar, S., Mohanty, A. K., Pavulraj, S., Bera, B. C., Vaid, R. K., Ahlawat, U. and Tripathi, B. N. 2020. Phage therapy for treatment of virulent Klebsiella pneumoniae infection in a mouse model. *Journal of Global Antimicrobial Resistance* 21: 34–41. https://doi.org/10.1016/J.JGAR.2019.09.018

Chow, M. S. and Rouf, M. A. 1983. Isolation and partial characterization of two *Aeromonas hydrophila* bacteriophages. *Applied and Environmental Microbiology* 45 (5): 1670–1676. https://doi.org/10.1128/AEM.45.5.1670-1676.1983

Dang, T. H. O., Xuan, T. T. T., Duyen, L. T. M., Le, N. P. and Hoang, H. A. 2021. Protective efficacy of phage PVN02 against *haemorrhagic septicaemia* in striped catfish *Pangasianodon hypophthalmus* via oral administration. *Journal of Fish Diseases* 44 (8): 1255–1263. https://doi.org/10.1111/jfd.13387

Deekshit, V. K., Maiti, B., Krishna Kumar, B., Kotian, A., Pinto, G., Bondad-Reantaso, M. G., Karunasagar, I. and Karunasagar, I. 2023. Antimicrobial resistance in fish pathogens and alternative risk mitigation strategies. *Wiley Online Library* 15 (1): 261–273. https://doi.org/10.1111/RAQ.12715

Dien, L. T., Ky, L. B., Huy, B. T., Mursalim, M. F., Kayansamruaj, P., Senapin, S., Rodkhum, C. and Dong, H. T. 2021. Characterization and protective effects of lytic bacteriophage pAh6.2TG against a pathogenic multidrug-resistant *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). *Transboundary and Emerging Diseases*. https://doi.org/10.1111/tbed.14321

Easwaran, M., Dananjaya, S. H. S., Park, S. C., Lee, J., Shin, H. J. and De Zoysa, M. 2017. Characterization of bacteriophage pAh-1 and its protective effects on experimental infection of *Aeromonas hydrophila* in Zebrafish (*Danio rerio*). *J Fish Dis* 40 (6): 841–846. https://doi.org/10.1111/jfd.12536

Hoang, H. A., Yen, M. H., Ngoan, V. T., Nga, L. P. and Oanh, D. T. H. 2018. Virulent bacteriophage of *Edwardsiella ictaluri* isolated from kidney and liver of striped catfish *Pangasianodon hypophthalmus* in Vietnam. *Dis Aquat Organ* 132 (1): 49–56. https://doi.org/10.3354/dao03302

Isyagi-Levine, N. and Fejzic, N. 2021. Aquatic Animal Health Monitoring Control and Surveillance in Uganda-Gap Analysis. Prepared by AGT International in Association With AquaBioTech Group. https://www.researchgate.net/profile/NihadFejzic/publication/373113738Promoting_Environmentally_Sustainable_Commercial_AquacultureProject_in_Uganda_Ministry_of_Agriculture_Animal_Industryand_Fisheries_aquatiAnimal_Health_Monitoring_Control_and_Surveillance_inUganda/links/64da619a25837316ee100b26/Promoting-Environmentally-Sustainable-Commercial-Aquaculture-Project-in-Uganda-MINISTRY-OF

AGRICULTURE-ANIMAL-INDUSTRY-AND-FISHERIES-Aquatic-Animal-Health-Monitoring-Control-and-Surveillance-in-Uganda.pdf

Jepkurui,M. 2023. Isolation and characterization of therapeutic lytic Phages against Multi-Drug resistant *Enterobacter Cloacae* in Kenya. https://www.researchgate.net/profile/Ivy-Mutai/publication/390842566_ISOLATION_AND_CHARACTERIZATION_OF_THERAPEUTIC_LYTIC_PHAGES_AGAINST_MULTIDRUG_RESISTANT_ENTEROBACTER_CLOACAE_IN_KENYA/links/68001ab2df0e3f544f4261da/ISOLATION-AND CHARACTERIZATION-OF-THERAPEUTIC-LYTIC-PHAGES-AGAINST-MULTI- DRUG-RESISTANT-ENTEROBACTER-CLOACAE-IN-KENYA.pdf

Jun, J. W., Kim, H. J., Yun, S. K., Chai, J. Y. and Park, S. C. 2015. Genomic structure of the Aeromonas bacteriophage pAh6-C and its comparative genomic analysis. *Arch Virol*, 160 (2): 561–564. https://doi.org/10.1007/s00705-014-2221-1

Kiggundu, R., Waswa, J.P., Konduri, N., Kasujja, H., Murungi, M., Vudriko, P., Akello, H., Lugada, E., Muiva, C., Were, E. and Tjipura, D. 2024. A One Health approach to fight antimicrobial resistance in Uganda: implementation experience, results, and lessons learned. *Biosafety and Health* 6 (2):125-132. https://www.sciencedirect.com/science/article/pii/S259005362400003X

Kim, J. H., Son, J. S., Choi, Y. J., Choresca Jr., C. H., Shin, S. P., Han, J. E., Jun, J. W. and Park, S. C. 2012. Complete genome sequence and characterization of a broad-host range T4-like bacteriophage phiAS5 infecting *Aeromonas salmonicida* subsp. salmonicida. *Vet Microbiol*, 157 (1– 2): 164–171. https://doi.org/10.1016/j.vetmic.2011.12.016

Kim, J. H., Son, J. S., Choi, Y. J., Choresca, C. H., Shin, S. P., Han, J. E., Jun, J. W., Kang, D. H.,Oh, C., Heo, S. J. and Park, S. C. 2012. Isolation and characterization of a lytic Myoviridae bacteriophage PAS-1 with broad infectivity in *Aeromonas salmonicida*. *Curr Microbiol* 64 (5) :418–426. https://doi.org/10.1007/s00284-012-0091-x

Luong, T., Salabarria, A.C., Edwards, R.A. and Roach, D.R. 2020. Standardized bacteriophage purification for personalized phage therapy. *Nature Protocols* 15 (9):2867-2890. https://doi.org/10.1038/s41596-020-0346-0

Maalej, S., Gdoura, R., Dukan, S., Hammami, A. and Bouain, A. 2004. Maintenance of pathogenicity during entry into and resuscitation from viable but nonculturable state in *Aeromonas hydrophila* exposed to natural seawater at low aeromonas. *Wiley Online Library* 97(3): 557– 565. https://doi.org/10.1111/j.1365-2672.2004.02336.x

Maldonado-Miranda, J.J., Castillo-Pérez, L.J., Ponce-Hernández, A. and Carranza-Álvarez, C. 2022. Summary of economic losses due to bacterial pathogens in aquaculture industry pp. 399-417. In: Bacterial fish diseases. Academic Press. Elsevier. Retrieved 30 June 2025, from https://www.sciencedirect.com/science/article/pii/B9780323856249000233

Nachimuthu, R., Royam, M.M., Manohar, P. and Leptihn, S. 2021.Application of bacteriophages and endolysins in aquaculture as a biocontrol measure. *Biological Control* 160:104678.

Nagel, T., Musila, L., Muthoni, M., Nikolich, M., Nakavuma, J.L. and Clokie, M.R. 2022. Phage banks as potential tools to rapidly and cost-effectively manage antimicrobial resistance in the developing world. *Current Opinion in Virology*M 53 :101208. Elsevier. Retrieved 30 June 2025, from https://www.sciencedirect.com/science/article/pii/S1879625722000153

Paquet, V. E., Vincent, A. T., Moineau, S. and Charette, S. J. 2019. Beyond the A-layer: adsorption of lipopolysaccharides and characterization of bacteriophage-insensitive mutants of *Aeromonas salmonicida* subsp. *salmonicida*. *Mol Microbiol* 112 (2): 667–677. https://doi.org/10.1111/mmi.14308

Pereira, C., Costa, P., Pinheiro, L., Balcão, V. M., and Almeida, A. 2021. Kiwifruit bacterial canker: an integrative view focused on biocontrol strategies. *Planta* 253 (2). https://doi.org/10.1007/s00425-020-03549-1

Schulz, P., Pajdak-Czaus, J., Robak, S., Dastych, J. and Siwicki, A. K. 2019. Bacteriophage-based cocktail modulates selected immunological parameters and post-

challenge survival of rainbowtrout (*Oncorhynchus mykiss*). *J Fish Dis* 42 (8): 1151–1160. https://doi.org/10.1111/jfd.13026

Sieiro, C., Areal-Hermida, L., Pichardo-Gallardo, Á., Almuiña-González, R., De Miguel, T., Sánchez, S., Sánchez-Pérez, Á. and Villa, T.G. 2020. A hundred years of bacteriophages: Can phages replace antibiotics in agriculture and aquaculture? *Antibiotics*. Mdpi.Com. 9 (8):493. https://www.mdpi.com/2079-6382/9/8/493

Teplitz, E.M., Mwainge, V.M., Wacira, T.N., Ogwai, C., Mayianda, M.J., Ochieng, L., Patel, E., Getchell, R., Mulanda, C.A. and Fiorella, K.J. 2025. Disease and antimicrobial resistance surveillance for Nile Tilapia pathogens in Lake Victoria, Kenya. Aflatoxins. Papers.Ssrn.Com. Retrieved 30 June 2025 from https://papers.ssrn.com/sol3/papers.cfm?abstract_id=5222661

Tokman, J.I., Kent, D.J., Wiedmann, M. and Denes, T., 2016. Temperature significantly affects the plaquing and adsorption efficiencies of Listeria phages. *Frontiers in Microbiology* 7:631. https://doi.org/10.3389/FMICB.2016.00631

Warburton, D. W., McCormick, J. K. and Bowen, B. 1994. Survival and recovery of *Aeromonas hydrophila* in water: Development of methodology for testing bottled water in Canada. *Canadian Journal of Microbiology* 40 (2): 145–148. https://doi.org/10.1139/M94-023

Żaczek, M., Weber-Dabrowska, B. and Górski, A. 2020. Phages as a cohesive prophylactic and therapeutic approach in aquaculture systems. *Antibiotics* 9 (9): 1–18. MDPI AG. https://doi.org/10.3390/antibiotics9090564