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ENGINEERING AND TECHNOLOGY
COLLOQUIUM



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PREFACE

Welcome to the 2nd International Science, Engineering and Technology Colloquium 2025 (ISETC 2025)!

It is with great pleasure that we present this year's colloquium, a distinguished gathering of scholars, researchers, and professionals dedicated to the advancement of applied sciences. Held virtually on 9th July 2025, ISETC 2025 serves as a platform to highlight cutting-edge research, foster meaningful networking, encourage collaborations, and explore innovative solutions to global challenges.

This year's theme, "The Power of Scientific Innovation: Intelligent Solutions for a Sustainable World" highlights our shared goal to solve global challenges through applied science. It celebrates innovation as a key to building a more sustainable and resilient future. We're proud to feature keynote speakers and presenters who bring fresh insights, bold ideas, and breakthroughs shaping tomorrow's world.

We would like to extend our heartfelt appreciation to the Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, for their outstanding support and assistance throughout the planning and execution of the event. Their dedication has been instrumental to the success of this colloquium. We would like to sincerely thank all participants and researchers for their valuable contributions. We also appreciate the reviewers for their careful evaluations and helpful feedback. Your hard work and support mean a lot to us.

As editors, we are proud to present this collection of proceedings. It is an honour to bring together the interesting and meaningful work of our speakers and authors. We hope the ideas shared will lead to new discoveries, encourage more research, and help solve global challenges through science and innovation.

Warm regards,

Dr. Emi Norzehan Mohamad Mahbob

Dr. Saiyidah Nafisah Hashim

Ms. Lili Widarti Zainuddin

Ms. Nurul Nazwa Mohammad

Ms. Rozaina Saleh

ISETC 2025 Proceedings Editors

SERUM IGM RESPONSE, HEPATIC VIABILITY, AND SURVIVAL OF TILAPIA IN KPP5 PHAGE-*Klebsiella pneumoniae* KP001 INTERACTION

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Abstract: Bacterial infections caused by *Klebsiella pneumoniae* pose a growing threat to aquaculture, particularly in red hybrid tilapia (*Oreochromis* spp.) farming. This study evaluates the therapeutic potential of *K. pneumoniae* Phage 5 (KPP5) in enhancing fish survival, controlling *K. pneumoniae* strain 001 (KP001) burden in the liver, and modulating immunoglobulin M (IgM) levels. Red hybrid tilapia were experimentally challenged with a median lethal dose (LD₅₀) of KP001 and subsequently treated with purified KPP5 at a multiplicity of infection (MOI) of 1.0. The phage-treated group exhibited lesser mortality rate (35%) compared to 60% mortality rate in the untreated infected group, with a relative percentage survival (RPS) of 41.67%. Hepatic analysis revealed complete bacterial clearance in treated fish at all time points, while untreated fish showed high bacterial loads and delayed clearance. Concurrently, hepatic phage titres remained higher in infected fish than in phage-only control, indicating sustained in vivo replication of KPP5 in the presence of its bacterial host. IgM concentrations in serum was markedly elevated in the phage-treated group, suggesting enhanced adaptive immune responses. These findings support the use of KPP5 as a viable and effective alternative to antibiotics, promoting fish survival while contributing to immune resilience and bacterial control in aquaculture settings. Future studies should focus on investigating long-term immune effects.

Keywords: Bacteriophage, immunomodulatory aquaculture, tilapia, pathogenic bacteria

INTRODUCTION

The red hybrid tilapia (*Oreochromis* spp.) plays a significant role in Malaysia's aquaculture sector, contributing approximately RM334 million annually to the industry (MAFS, 2022). However, the species faces increasing vulnerability to bacterial pathogens such as *Aeromonas hydrophila* (Basri et al., 2020), *Streptococcus agalactiae* (Lukman et al., 2023; Phuoc et al., 2021; Luo et al., 2018), and particularly *Klebsiella pneumoniae* (Vaneci-Silva et al., 2022) which is an emerging pathogen known for its virulence, biofilm production, and multidrug resistance (Liao et al., 2023; Alfonso et al., 2022; Vaneci-Silva et al., 2022). Outbreaks of *K. pneumoniae* have caused massive losses in tilapia farms, including one event in Brazil where 90,000 fish died within four days (Vaneci-Silva et al., 2022). With the bacteria being resistant to antibiotics like penicillin and ampicillin (Thongkao & Sudjaroen, 2019), alternative treatments seem imperative.

Phage therapy, which operates through the lytic cycle to kill bacteria, has been shown to be highly specific, environmentally friendly, and effective even against antibiotic-resistant strains (Bae et al., 2024; Kasman & Porter, 2024; Mishra et al., 2024). In fish, phages can also stimulate both innate and adaptive immune responses. Specifically, immunoglobulin M (IgM), the principal antibody in teleosts, plays a central role in humoral immunity (Yin et al., 2019). Elevated IgM levels following phage exposure indicate activation of systemic immune defences that may enhance host resilience to infection (Dien et al., 2022). Additionally, liver viability analysis helps determine whether phage treatment successfully clears infection at the tissue level while maintaining phage persistence which is a critical factor for ensuring therapeutic success.

This study explores on assessing the safety and efficacy of purified *K. pneumoniae* Phage 5 (KPP5) against *K. pneumoniae* strain 001 (KP001) in red hybrid tilapia. The objectives are threefold: (1) to evaluate fish survival following infection and phage treatment, (2) to investigate the hepatic viability of both KP001 and KPP5 over time,

and (3) to examine the changes in IgM concentration as an indicator of adaptive immune activation. The main question this study tries to answer is whether phage therapy can really clear out harmful bacteria inside the fish without compromising their immune system or causing other chronic complications. A big part of this research is about figuring out how well the phages persist around in the liver and how the fish's IgM levels respond to the treatment. By focusing on fish survival, liver bacterial and phage counts, and immune responses, this study aims to offer useful insight into how phage therapy could contribute critical insights into the real-world fish farming, especially as antibiotic resistance becomes a bigger issue.

MATERIALS AND METHODS

This study was conducted under the ethical approval of the Institutional Animal Care & Use Committee, Universiti Malaya (G8/10032025/07012025-02/R). Red hybrid tilapia (*Oreochromis* spp.), averaging 14.7 ± 4.4 grams in weight, were acclimatised for two weeks in 500 L tanks maintained at $25.1 \pm 0.08^\circ\text{C}$ under a 12:12-hour light-dark cycle. Prior to experimental use, fish were screened for bacterial contamination using XLD agar, ensuring they were free from infection. The bacterial pathogen *Klebsiella pneumoniae* strain KP001 (PV608403.1), originally isolated from the eye of a diseased *Pangasius hypophthalmus*, was used for challenge studies. *K. pneumoniae* Phage 5 (KPP5), isolated from river water collected at the same location, was purified through 14 rounds of the double agar overlay method, resulting in a high-titre lysate of 3.60×10^9 PFU/mL.

To assess pathogenicity, fish were intraperitoneally injected with varying concentrations of KP001 (10^7 – 10^9 CFU/mL), and mortality was monitored over 10 days. The median lethal dose (LD_{50}) was calculated as 7.8×10^6 CFU/fish using probit analysis. For the treatment trial, 160 fish were divided into four groups: uninfected and untreated (Group A), infected but untreated (Group B), infected with KP001 and treated with KPP5 (Group B), and treated with KPP5 only (Group D). Each group consisted of two replicate tanks with 20 fish each. Phage treatment was administered intraperitoneally at an MOI of 1.0 shortly after bacterial challenge. Fish mortality was observed for seven days, and relative percentage survival (RPS) was calculated to determine treatment effectiveness. Both studies were done in duplicates.

To evaluate hepatic bacterial and phage viability, liver tissues were sampled at 6, 72, and 168 hours post-infection (HPI). A 0.1 g portion of liver tissue from each fish was homogenised in phosphate-buffered saline (PBS, pH 7.4), centrifuged, and analysed to determine CFU/mL of KP001 and PFU/mL of KPP5. For serum IgM analysis, blood was drawn from the caudal vein, allowed to clot, and serum was extracted after centrifugation. IgM levels were measured using a competitive ELISA kit (Cusabio, USA) specific to fish IgM, with optical density readings taken at 450 nm and compared against a standard curve to determine IgM concentrations in $\mu\text{g/mL}$.

RESULTS AND DISCUSSION

The application of *Klebsiella pneumoniae* Phage 5 (KPP5) significantly improved the survival of tilapia infected with *K. pneumoniae* strain 001 (KP001). Figure 1 shows fish treated with KPP5 (Group C, GC) exhibited a 35% mortality rate, while those in the infected but untreated group (Group B, GB) experienced 60% mortality within seven days. The phage-only (Group D, GD) and uninfected control (Group A, GA) groups showed no deaths. These differences were statistically significant, with a RPS of 41.67% in the treated group, supporting the findings of Luo et al. (2018), who reported similar protective effects of phage therapy against *S. agalactiae* in tilapia.

Hepatic bacterial viability analysis further supported the efficacy of KPP5, as shown in Table 1. In Group C, complete clearance of KP001 was observed at 6-, 72-, and 168-hours post-infection (HPI). In contrast, the untreated infected group showed a high bacterial load at 6-HPI (2.74×10^8 CFU/mL), a drop to zero at 72-HPI, and a rebound to 1.80×10^5 CFU/mL at 168-HPI, indicating that the bacteria were able to persist and possibly recolonise in the absence of phage intervention. These findings are consistent with the work of Vaneci-Silva et al. (2022), who described the resilience and re-emergence of *K. pneumoniae* in infected tilapia populations, and highlight the importance of continuous phage activity to prevent bacterial resurgence.

Meanwhile, hepatic phage viability demonstrated that KPP5 replicated effectively in vivo when its host bacteria were present, as shown in Table 2. Phage titres in Group C remained consistently higher across all time points than in Group D, beginning at 1.15×10^8 PFU/mL at 6-HPI and declining to 4.00×10^2 PFU/mL by 168-HPI. This indicates that phage replication was sustained in the presence of the target pathogen, in line with the auto-dosing principle described by Abedon (2016). In contrast, the more rapid phage decline in Group D supports earlier observations that phages cannot amplify without their bacterial hosts (Mishra et al., 2024).

The immune-modulating effects of KPP5 were evident in the IgM concentrations measured in serum, where the phage-treated fish showed significantly elevated levels compared to untreated infected fish (Figure 2). This increase was most notable at 168-HPI. The finding aligns with studies by Dien et al. (2022), which highlight IgM as a central component of the adaptive immune response in teleosts, particularly following microbial or phage exposure. The rise in IgM suggests that phage therapy may not only reduce pathogen burden but also stimulate protective humoral immunity, enhancing the overall resistance of the fish to future infections.

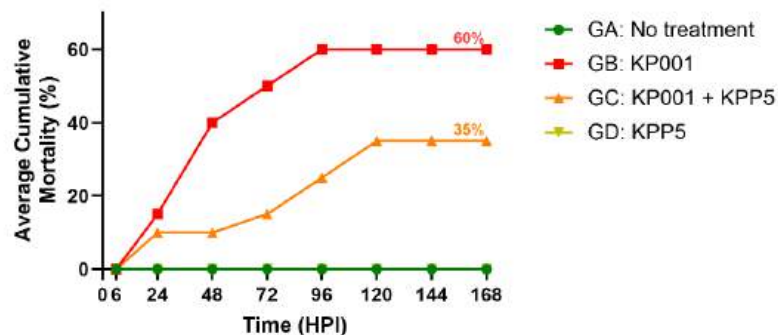


Figure 1. Average cumulative mortality (%) of red hybrid tilapia (*Oreochromis* spp.) over 168 hours post-infection (HPI) under the duplicates of four treatment conditions. Fish were administered no treatment (GA, green circles), *Klebsiella pneumoniae* strain 001 (KP001) (GB, red squares), KP001 + *Klebsiella pneumoniae* Phage 5 (KPP5) (GC, orange triangles), or KPP5 alone (GD, moss green inverted triangles). Group B exhibited the highest mortality (60%), followed by Group C (35%). No mortality was observed in Groups A and D, indicating the pathogenicity of KP001 and a potential protective effect of phage KPP5.

Table 1. Hepatic Bacterial Viability (CFU/mL) of *Klebsiella pneumoniae* strain 001 (KP001) in Group B and Group C at 6-, 72-, and 168-Hours Post-Infection (HPI)

HPI	Group B (KP001) (CFU/mL)	Group C (KP001 + KPP5) (CFU/mL)	95% CI	Significant difference
6	2.74×10^{8a}	0^b	254221828 to 293778172	Yes
72	0^b	0^b	-19778172 to 19778172	No
168	1.80×10^{5b}	0^b	-19598172 to 19958172	No

Means in the same row with different superscript letters indicate statistically significant differences ($P < 0.05$). Significance was determined using Tukey's post hoc test following two-way repeated measures ANOVA.

Bacterial counts were measured in the liver to assess the efficacy of phage treatment. Group B (KP001) showed a high bacterial load at 6 HPI, followed by complete clearance at 72 HPI and a resurgence at 168 HPI. In contrast, Group C (KP001 + KPP5) exhibited complete bacterial clearance at all time points. Statistical analysis revealed a significant reduction in bacterial viability in Group C compared to Group B at 6 HPI ($P < 0.0001$), indicating rapid bactericidal activity of phage KPP5. No significant differences were observed at 72 and 168 HPI. Significance was determined using adjusted p-values with a threshold of $P < 0.05$.

Table 2. Hepatic phage viability (PFU/mL) of *Klebsiella pneumoniae* Phage 5 (KPP5) in Group C and Group D at 6-, 72-, and 168-Hours Post-Infection (HPI)

HPI	Group D (KPP5) (PFU/mL)	Group C (KP001 + KPP5) (PFU/mL)	95% CI	Significant difference
6	6.51 x 10 ^{5a}	1.15 x 10 ^{8b}	-2.660 to -1.833	Yes
72	1.99 x 10 ^{3a}	1.76 x 10 ^{5b}	-2.499 to -1.396	Yes
168	2.00 x 10 ^{2a}	4.00 x 10 ^{2b}	-	Yes

Means in the same row with different superscript letters indicate statistically significant differences ($P < 0.05$). Significance was determined using Tukey's post hoc test following two-way repeated measures ANOVA.

Phage titres were quantified in the liver to evaluate replication dynamics in the presence or absence of host bacteria. Group C (KP001 + KPP5) consistently exhibited higher phage titres compared to Group D (KPP5), with significant differences observed at all time points ($P < 0.05$). These findings suggest that phage replication was supported in the presence of its bacterial host (Group C), while titres declined in Group D due to the absence of bacterial amplification. Significance was determined using adjusted p-values with a threshold of $P < 0.05$.

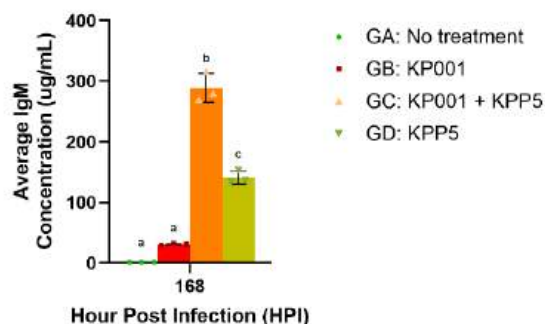


Figure 2. Serum immunoglobulin M (IgM) concentrations in red hybrid tilapia (*Oreochromis* spp.) at 168-hours post-infection (HPI) across four treatment groups: No treatment (GA, green circles), *Klebsiella pneumoniae* strain 001 (KP001) (GB, maroon squares), KP001 + *Klebsiella pneumoniae* Phage 5 (KPP5) (GC, cream triangles), or KPP5 alone (GD, green inverted triangles). Data are presented as mean $\pm$ standard error (SE) ($n = 3$ per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. Different letters represent significant differences between groups. The combination treatment (Group C) elicited the highest IgM response, followed by KPP5 alone (Group D), indicating a strong immunostimulatory effect of phage therapy.

CONCLUSIONS

This study demonstrates that *Klebsiella pneumoniae* Phage 5 (KPP5) effectively enhances survival and immune response in red hybrid tilapia infected with *Klebsiella pneumoniae* strain 001 (KP001), supporting its dual role as a targeted antibacterial agent and immune modulator. The complete bacterial clearance and elevated IgM levels in treated fish highlight the therapeutic potential of the phage, while the observed resurgence of bacteria in untreated fish underscores the importance of sustained phage activity. These findings suggest that phage therapy offers a viable, environmentally friendly alternative to antibiotics in aquaculture. Future work should explore long-term immune effects, optimal dosing strategies, and field applications to ensure broader efficacy and integration into fish health management systems.

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Surat kami : 700-KPK (PRP.UP.1/20/1)
Tarikh : 20 Januari 2023



Prof. Madya Dr. Nur Hisham Ibrahim
Rektor
Universiti Teknologi MARA
Cawangan Perak

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Kelulusan daripada pihak tuan dalam perkara ini amat dihargai.

Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Saya yang menjalankan amanah,

Setuju.

27.1.2023

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PROF. MADYA DR. NUR HISHAM IBRAHIM
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