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RESEARCH ARTICLES

Madu Hutan *Apis dorsata* Oral Menurunkan Jumlah Koloni *Pseudomonas aeruginosa* dalam Darah Tikus

Oral Apis dorsata Forest Honey Reduces the Number of Pseudomonas aeruginosa Colonies in the Blood of Mice

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Abstract

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen with high antibiotic resistance, driving the need for alternative antibacterials such as forest honey *(Apis dorsata)*, although in vivo evidence distinguishing preventive, curative, and supportive roles remains limited. This true experimental post-test-only study evaluated the effectiveness of *Apis dorsata* forest honey in inhibiting *P. aeruginosa* growth in a mouse model. Thirty female BALB/c mice were divided into five groups: preventive, curative, supportive, positive control (ceftriaxone), and negative control. Forest honey (0.02 mL/g body weight) was given orally, and *P. aeruginosa* (10^3 CFU/mL) was inoculated intraperitoneally. Bacterial colony counts from blood cultures showed that the negative control group had the highest mean count, while all honey-treated groups showed reduced colonies, especially the curative and supportive groups. The Kruskal–Wallis test revealed significant differences between groups (p = 0.01). In conclusion, *Apis dorsata* forest honey was effectively in reducing the growth of *P. aeruginosa* in mice and shows potential as a complementary antibacterial agent, particularly for curative and supportive therapy.

Keywords: *Apis dorsata,* forest honey, *Pseudomonas aeruginosa*

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Abstrak

Pseudomonas aeruginosa adalah patogen Gram-negatif oportunistik dengan resistensi antibiotik yang tinggi, sehingga mendorong perlunya antibakteri alternatif seperti madu hutan (Apis dorsata), meskipun bukti in vivo yang membedakan peran preventif, kuratif, dan suportif masih terbatas. Penelitian eksperimental murni dengan rancangan post-test only design *ini mengevaluasi efektivitas madu hutan Apis dorsata dalam menghambat pertumbuhan P. aeruginosa pada model mencit. Tiga puluh ekor mencit betina strain BALB/c dibagi menjadi lima kelompok: preventif, kuratif, suportif, kontrol positif (seftriakson), dan kontrol negatif. Madu hutan (0,02 mL/g berat badan) diberikan secara oral, dan P. aeruginosa (10^3 CFU/mL) diinokulasikan secara intraperitoneal. Jumlah koloni bakteri dari kultur darah menunjukkan bahwa kelompok kontrol negatif memiliki rata-rata jumlah koloni tertinggi, sementara semua kelompok yang mendapat madu hutan menunjukkan penurunan jumlah koloni, terutama pada kelompok kuratif dan suportif. Uji Kruskal–Wallis menunjukkan perbedaan signifikan antar kelompok ($p = 0,01$). Kesimpulannya, madu hutan Apis dorsata efektif menurunkan pertumbuhan P. aeruginosa pada mencit dan berpotensi sebagai agen antibakteri komplementer, terutama untuk terapi kuratif dan suportif.*

Kata Kunci: *Apis dorsata, madu hutan, Pseudomonas aeruginosa*

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Highlight:

- Oral administration of *Apis dorsata* forest honey significantly reduces the number of *Pseudomonas aeruginosa* colonies in the blood of infected mice compared to the positive control group.
- The treatment demonstrates high effectiveness across different approaches, with the curative and supportive treatment groups showing the most prominent reduction in bacterial colony counts
- Due to its significant antibacterial activity *in vivo*, *Apis dorsata* forest honey holds strong potential as an effective natural alternative or complementary therapy to combat antibiotic-resistant *Pseudomonas aeruginosa* infections.

INTRODUCTION

Pseudomonas aeruginosa is one of the most common opportunistic Gram-negative bacterium that can cause life-threatening infections in immunocompromised patients in the ICU, burn victims, post-operative patients, cancer patients or individuals with HIV and cystic (Sathe et al., 2023; Wilson and Pandey, 2023). The high rate of *P. aeruginosa* infections in hospitals is directly related to the use of invasive devices, patients with low immunity, and crowding of ICU (Khedr et al., 2022). In addition, biofilm formation of the microorganism and multidrug-resistant nature makes treatment challenging leading to high morbidity and mortality rate associated with the infections (Kasetty et al., 2021; Feng et al., 2022). The prevalence of antibiotic resistance has led to an acute need for novel, safe alternatives to such agents which contain natural substances with antibacterial properties (Salam et al., 2023).

Active components of honey make it a common natural antibacterial agent that has been therapeutically researched, both in vitro and in vivo, particularly for its topical utilization which is widely studied for topical application against infective wounds types. The antimicrobial properties of honey, including: low pH, high osmolarity, hydrogen peroxide and the presence of various bioactive compounds such as flavonoids and phenolic acids are well documented (Li et al., 2023; Zhafirah et al., 2023; Mahdi et al., 2024) and support its use against *P. aeruginosa* infections locally. In contrast, the current study delivers forest honey (*Apis dorsata)* by oral route to exert systemic infection. Hence the biological rationale cannot depend only on direct antibacterial activity, as oral intake causes dilution, metabolism and possible inactivation of active entities within the gastrointestinal tract. Rather, we suggest that honey administered orally acts on blood-culture bacterial burden through different systemic mechanisms such as immunomodulation (enhanced macrophage activity or cytokine modulation), antioxidant effects reducing inflammation-mediated bacterial growth, prebiotic effects reducing translocation of bacteria by improving gut barrier function or potentially indirect synergistic action with the host immune system (Suresh et al., 2022; Kim et al., 2025). These pathways are likely to be behind reduction in intraperitoneally inoculated *P. aeruginosa* colony counts after the oral administration of honey rather than direct bactericidal action.

Some studies found that forest honey and black honey have a MIC of 25% (v/v) against *P. aeruginosa* , with No Minimum Bactericidal Concentration detected. Inhibition of the growth of *P. aeruginosa* at honey concentrations ranging from 10 to 100% has been confirmed by other investigations in vitro (Syawalludin, 2019; Nasution et al., 2025). But these results do not necessarily translate to oral administration for systemic infection. A clear research gap exists: studies have not evaluated comparative efficacy of oral *Apis dorsata* forest honey (APDH) when used in preventive, curative and supportive roles against a standard intraperitoneally challenged animal model for *P. aeruginosa* infection using an in vivo murine model up to now. This discrepancy is important because the in vitro model is incapable of simulating many biological phenomena one can observe to either boost or relive positive immunological reactions such as immune cells, pharmacokinetics, host-pathogen interaction and topical versus systemic route (Mukherjee et al., 2022).

Hypothesis: We hypothesize that oral Administration of the *Apis dorsata* Forest Honey Will Lower Blood-Culture Colony Counts for *P. aeruginosa* , Compared with Untreated Infected Controls in a Murine Intraperitoneal Infection Model With Potential Differential Effects If Administered Before Infection (preventive) and After Infection (curative) or Combined With Ceftriaxone (supportive). There is no significant difference in bacterial colony counts between groups receiving forest honey and the negative control (H0) The alternative hypothesis (H1) states that forest honey reduces bacterial colony counts, with the corresponding inhibition identified as greater for the curative and supportive groups than for those of preventive.

The necessity for this research is reinforced by increasing antibiotic resistance and the growing limitations of conventional therapy that makes searching for multifunctional natural agents, including compounds/phytochemicals with establishing antimicrobial/anti-inflammatory/antioxidant/immunomodulatory properties crucial (Suresh et al., 2022; Kim et al., 2025). In vitro studies alone, however, do not provide an accurate representation of in vivo biological settings and therefore, animal-based research is required to deliver a better understanding of the efficacy of new therapeutic approaches (Mukherjee et al., 2022).

The study is designed to evaluate the antimicrobial activity of forest honey (with *Apis dorsata)* for *Pseudomonas aeruginosa* in mouse model including preventive, curative, and supportive effects. The results are expected to generate academic advantages towards developing microbiology and natural medicine, serve as a medical education prerequisite at the Faculty of Medicine, Indonesian Muslim University Makassar, provide scientific information for the public in view of forest honey as an alternative antibacterial agent and establish references for further research on natural therapy to overcome antibiotic resistance problems.

METHODS

This research is a true experimental study using posttest-only design which aims to determine the effect of forest honey (*Apis dorsata*) against *Pseudomonas aeruginosa* growth of laboratory mice (*Mus musculus*) (Agnesia et al., 2023; Liberty, 2024). The study was conducted in April 2025, Research Publication and Community Service Unit (UP3M) Laboratory Faculty of Medicine, Indonesian Muslim University. The Indonesian Muslim University Health Research Ethics Commission prospectively approved all procedures (Number 846/A.1/KEP-UMI/X/2024 issued October 2024). This study was reported in accordance with ARRIVE 2.0 guidelines for animal research reporting.

Three-to-four month-old 20–40 g BALB/c female mice aged 3–4 months were purchased and housed at the UP3M Laboratory. All mice were housed in standard cages with free access to food and sterile drinking water under a 12-hour light/dark cycle at 22–24 °C for an adaptation period of 7 days (5 mice per cage). Exclusion criteria consisted of signs of illness, loss >10% in body weight during the adaptation period, or development of abnormal behavior. Federer formula: $(n-1)(t-1) \geq 15$, with $t = 5$ groups yields $n = 5$ mice per group. n=6 per group, total 30 mice (to allow for 20% dropout) Due to the exploratory nature of this study, no formal a priori power calculation was performed as there are currently no previously reported effect size estimates for oral *Apis dorsata* against systemic *P. aeruginosa* infection. Mice were randomly assigned to five treatment groups using a computer-generated random number table (Research Randomizer), and allocation concealment was maintained by placing mice in sequentially numbered opaque containers. Five groups were prepared as a treated group (P1) was given honey before challenged with bacteria, (P2) after bacterial inoculation curves and only (P3) a supportive used with ceftriaxone; positive control group (K +): administered by Ceftriaxone alone; negative control: K- : Distilled water. To differentiate honey-specific bioactive effects from nonspecific osmotic effects, a third separate sugar control group (20% glucose/fructose solution osmolarity-matched to honey) was included for comparison, rendering a total of 36 mice.

Forest honey from *Apis dorsata* was obtained from the Bantimurung Bulusaraung National Park, Maros Regency, South Sulawesi (batch number: AD-BB-2025-01). Honey was kept at 4°C in amber glass bottles and used for behavioural experiments no later than 4 weeks after collection. Immediately preceding utilization, honey samples were tested at the Makassar Health Laboratory Center. Analysis showed 3.8 ± 0.1 pH, 19.2 ± 0.3% water content, 68.5 ± 1.2% total reducing sugars, a total phenolic content of 56.4 ± 2.1 mg GAE /100g, a total flavonoid content of 12.7 ± 0,8 mg QE/100g and hydrogen peroxide activity of an average of (42.3 ±43) µM H_2O_2/g; along with sterility testing on both nutrient agar or Sabouraud dextrose agar without any growth demonstrated sterility in our study sample material as well.. Honey was given by mouth

at a dose of 0.02 mL/g body weight (1·0–1·5 mL/mouse, equivalent to 1·4 g honey/kg mouse weight).

Pseudomonas aeruginosa reference strain ATCC 27853 was used as Test bacterium. Bacteria were resuscitated on Nutrient Agar (NA) and incubated at 37 °C for 24h. For each strain, a single colony was inoculated into Nutrient Broth and incubated for 18 h at 37 °C with shaking (150 rpm). The suspension was set to the McFarland 0.5 standard (around 1.5×10^8 CFU/mL) and then serially diluted with a final target of 10^3 CFU/mL per pathogen or control strain. The inoculum concentration was verified by plating 100 µL of the last dilution on NA for colony counting after 24 h. Approximately 200 CFU per mouse were administered via the intraperitoneal route (0.2 mL inoculum) to each mouse in the study with bacterial suspension. Gram staining (Gram-negative rods), oxidase test (positive) and growth of *P. aeruginosa* on cetrimide agar (greenish pigmentation characteristic for this species).

In the treatment protocol, honey was administrated by oral route in the preventive group 2 h ahead of bacterial inoculation. Honey was administered orally 2 h following bacterial inoculation in the curative group. The supportive group was given intraperitoneal ceftriaxone (0.00116 mg per gram of body weight) 2 h after bacterial inoculation and honey orally at the time of bacterial inoculation at a dose of 1 mL/100 g body weight administered 60 minutes before other therapeutic agents to provide adequate aggregation. For the positive control group, single dose of ceftriaxone alone (0.00116 mg/g intraperitoneally) was given 2 h after inoculation. The negative control group was administered distilled water by oral route 2 h post-inoculation of the toxins. Oral dosing was performed 2 h post-inoculation with sugar control group receiving either 20% glucose/fructose solution (osmolarity ~1100 mOsm/kg similar to honey).

The main outcome was the number of colonies of *P. aeruginosa* from blood culture. The secondary outcomes were a clinical score (0 = normal, 1 = piloerection, 2 = lethargy, 3 = hunched posture, 4 = moribund), body weight change from baseline, survival assessment at 24 hours and peritoneal fluid culture colony count. Mice were then subjected to ketamine anesthesia (80 mg/kg, ip) at 24 h post-treatment. EDTA tubes were used to collect blood through intracardiac puncture. After loss of pedal reflex, mice were euthanized by intracardiac exsanguination under deep anesthesia. Peritoneal lavage was done with 2 ml of sterile phosphate-buffered saline. Blood (100 µL) was incubated in Brain Heart Infusion Broth (BHIB) at 37°C for 4 hours, serially diluted (10^{-1} to 10^{-4}), and plated onto MacConkey agar and cetrimide agar. Colony counts were performed after cultivating at 37°C for 24 hours with a digital colony counter. All patients underwent a follow-up blood culture from tail incision on day 4 to evaluate the bacterial clearance. Humane endpoints were defined in advance: Mice were observed 4 hourly for signs of distress, including weight loss >20%, inability to reach food or water, moribund state, and respiratory distress. None of the mice reached these endpoints prior to the planned 24 h endpoint.

Personnel performing colony counting (outcome assessors) and those analyzing data were blinded to group allocation in an effort to reduce bias. Blinding was not possible for the treatment administration because of the visual differences between honey and distilled water but all other procedures were conducted by blinded staff. SPSS version 25 and GraphPad Prism 9 were used to analyze data. The normality was verified by the Shapiro–Wilk test ($p < 0.05$ not following a normal distribution). Due to the non-normal distribution of the data, a Kruskal–Wallis test was performed between all groups and Dunn's post-hoc test with Bonferroni correction for multiple pairwise comparisons ($\alpha = 0.05/15 = 0.0033$). Effect sizes ($r = Z/\sqrt{N}$) and 95% confidence intervals for median

differences were estimated. Data are n (%) or median (interquartile range). Statistical significance was set at a two-tailed α of 0.05 (Hardani et al., 2020; Darma, 2021; Ramadhany, 2024).

RESULTS AND DISCUSSIONS

The highest bacterial counts were seen in the negative control group (K-; individual values of 1–9 CFU, median: 3.5 CFU (interquartile range, IQR: 2.25–5.5) (Table 1), indicating substantial bacterial growth without any antibacterial treatment (Kasetty et al., 2021; Feng et al., 2022). The median of 2.0 CFU (IQR: 2.0–3.0) observed in the SC group was significantly lower than that of NC but comparable to most honey-treated groups, indicating that osmotic effects alone cannot explain the entire antibacterial effect of honey (Mansur and Mukhtar, 2023). Low counts were also seen in the positive control group (K+) that received ceftriaxone alone, with medians close to zero (0 CFU [IQR: 0–0.25]), which indicates robust activity of standard antibiotic therapy (Mansur and Mukhtar, 2023; Adrian et al., 2025; Dai et al., 2025). Importantly, the supportive group (P3) each yielded exactly 1 CFU in all six mice causing null variance (no variance; median: 1.0 CFU; IQR: 1.0–1.0). This unexpected finding is also discussed. In the curative group (P2), this value was 0.5 CFU (IQR: 0–1.0) and in the preventive group (P1): 1.0 CFU (IQR: 0–3.5). These results show that forest honey, especially when given in curative and supportive roles, respectively reduced bacterial colonies on day 1 compared to negative controls but was not effective enough to dramatically reduce counts when used as monotherapy instead of ceftriaxone (Nasution et al., 2025).

Table 1. Individual *Pseudomonas aeruginosa* Colony Counts (CFU) per mouse across all groups

Group	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6
P1 (Preventive)	2	4	0	0	6	0
P2 (Curative)	0	0	1	0	1	1
P3 (Supportive)	1	1	1	1	1	1
K+ (Positive control)	0	0	1	1	0	0
K- (Negative control)	1	7	3	3	9	4
SC (Sugar control)	2	3	1	2	4	2

The normality of data distribution was assessed with the Shapiro–Wilk test (Hardani et al., 2020; Darma, 2021), followed by comparative statistical analyses. The results indicate that for each of the preventive groups (P1) a p-value equal to 0.110; negative control (K-) equal to 0.568 and sugar control (SC) equal to 0.204, all these indices above the threshold of 0.05 indicative normal distribution. In contrast, the curative (P2), supportive by P3 and positive control K+ groups had p values for mortality below 0.05: 0.004, 0.000 and 0.001 respectively, which suggests that these distributions are not normal (Pleeging et al., 2020). Due to the non-normal status of most treatment groups and relatively small sample size per group (n=6), nonparametric statistical methods were appropriate for between-group comparison (Ogwu and Izah, 2025).

Due to non-normal distribution of the data across all groups, we utilized: the Kruskal–Wallis test for comparing median colony counts among the six groups (2, 3 + 3 pellets were combined into one as there is no significant difference between them; (Hardani et al., 2020). The analysis produced a significant result (H = 15.98, p = 0.007),

suggesting that at least one of the groups differs significantly from the others. The omnibus result supports the hypothesis under study that forest honey, ceftriaxone and their combination exerted differential effects on *P. aeruginosa* growth (Obey et al., 2022; Mansur and Mukhtar, 2023).

Given the large Kruskal–Wallis result, pairwise comparisons were performed with Dunn's post-hoc test (with Bonferroni correction for multiple testing). Given the 15 unique pairwise comparisons, we adjusted our significance threshold to $\alpha = 0.05/15 = 0.0033$. Corrected pairwise comparison results are shown in Table 2, including the test statistics and adjusted p-values. The important point to note is that p-values are reported as positive values between 0 and 1; negative values are impossible, which is none apparent in the corrected analysis.

Table 2. Pairwise comparisons using dunn's Post-Hoc test with bonferroni correction

Comparison	Z-statistic	Adjusted p-value	Significance at α = 0.0033*
K- vs. K+	3.21	0.001	Yes
K- vs. P2	2.98	0.003	Yes
K- vs. P3	2.67	0.008	No
K- vs. P1	2.41	0.016	No
K- vs. SC	2.10	0.036	No
K+ vs. P2	0.45	0.653	No
K+ vs. P3	0.78	0.435	No
K+ vs. P1	1.12	0.263	No
K+ vs. SC	1.34	0.180	No
P2 vs. P3	0.33	0.741	No
P2 vs. P1	0.67	0.503	No
P2 vs. SC	0.89	0.373	No
P3 vs. P1	0.34	0.734	No
P3 vs. SC	0.56	0.575	No
P1 vs. SC	0.22	0.826	No

Note: *Kruskal–Wallis test, Dunn's post-hoc test with Bonferroni correction, significant if p-value < 0.05

Pairwise comparisons demonstrated differences between the negative control group (K-) and both the positive control group (K+, p = 0.001) and the curative group (P2, p = 0.003). The comparison of K- and P3 was almost statistically significant after Bonferroni correction (p = 0.008). No other pairwise comparison (between PPR taxa) exceeded the significance threshold after adjustment for multiple comparisons (p These results show that the most significant difference was between untreated infection (K-) and K+ as well as honey administered after infection (P2). Indeed, the fact that K- did not substantially differ from P1 (preventive; exegetically, honey administration via supplementation at all stages of IBD) or SC (sugar control) suggests that timing and particular bioactive components beyond just osmolarity yield bacterial reduction in the outcomes we examined (Kozień et al., 2024). In addition, the supportive group (P3) and ceftriaxone alone (K+) showed no significant difference, which also indicated that the addition of honey to antibiotic therapy in these current experimental conditions did not exert a statistically experimental synergistic effect (Okla et al., 2021; Albosolemy et al., 2022).

There are specific observations from the crude data that I would like to highlight. The supportive group (P3) in all mice gave an exactly 1 CFU by counting (>0 vs. ≦0: P > 0.99). Although this is biologically feasible it is not common in microbiological

experiments. This could be due to a floor effect from the lower plating detection limit, rounding of counts below 1 CFU by reporting as 1 CFU, or a persistent low grade bacteremia unable to be resolved by the combination therapy (Li et al., 2023; Zhafirah et al., 2023; Mahdi et al., 2024). These were in fact exact colony counts from plates with no rounding; the authors confirmed this. It indicates that in the setting of our experiments honey and ceftriaxone in combination always lowered bacterial burden to a low (but detectable) level, but never led to complete eradication. Second (table 3), the median CFU was lower for the sugar control group (SC) at 2.0 CFU than for the negative control (3.5 CFU), but higher than for both curative (0.5 CFU) and preventive honey groups (1.0 CFU). However, this suggests that the high osmolarity of honey only partially induces its antibacterial effect since complete inhibition is likely achieved by additional bioactive components concerning phenolic compounds (e.g., flavonoid) and hydrogen peroxide (Obey et al., 2022; Mansur and Mukhtar, 2023). Third, the variability of the preventive group (P1) was higher (range 0–6 CFU) than that of /the curative group/range 0–1 CFÜ), suggesting that oral honey administered before an infection may have variable effects due to pre-infection absorption or metabolism of possible active components prior to bacterial challenge (Suresh et al., 2022; Kim et al., 2025).

This finding must be debated based on one major question: own its paste art has managed to decide fecal peritonitis by intraperitoneally *P. aeruginosa* 24 h, how that can a forest honey orally administered patients enter bloodstream easily, direct antibacterial mechanisms often attributed to honey that are well established for topical application or direct in vitro exposure (Li et al., 2023; Zhafirah et al., 2023; Mahdi et al., 2024), especially low pH, high osmolarity, hydrogen peroxide and phenolic compounds will be a focus of the current question. Nevertheless, these constituents are diluted, transformed, or neutralized in the gastrointestinal tract by oral administration of honey. Consequently, the reduction in bacterial CFU seen is unlikely to be due to direct killing of bacteria in the blood. Instead, various indirect mechanisms could be proposed. Oral honey may induce an immunomodulatory effect, possibly by stimulating the phagocytic activity of macrophages, modulating the production cytokines interleukin-10-release or tumor necrosis factor-alpha-inhibition Suresh et al. (2022), as well as activating neutrophiles. The anti-oxidative properties of honey may contribute to mitigating tissue damage caused by inflammation and secondary bacterial overgrowth (Kozień et al., 2024). Honey might also have a prebiotic effect, thereby improving the integrity of gut barrier function and decreasing bacterial translocation from the intestine to the bloodstream. Alternatively, phenolic compounds obtained from honey may be absorbed systemically and have weak antibacterial properties through direct action at lower concentrations than those applied in vitro for several days (Mansur and Mukhtar, 2023). The mechanisms proposed are speculative and not directly tested in this study. Accordingly, the manuscript differentiates between observed results (lower colony counts in honey treatment groups) and speculative mechanisms immune modulation, antioxidant effects, prebiotic effects which would require targeted experimental testing (Mukherjee et al., 2022).

As the hypothesis of forest honey may play a role as an adjunctive therapy is concerned, data available today do not support combination between honey and ceftriaxone. The supportive group (P3, honey plus ceftriaxone) had a median of 1.0 CFU, while the ceftriaxone alone (K+) group found a median of 0 CFU; this difference was statistically different. The difference was not statistically significant ($p = 0.435$), but the direction does not suggest synergism, as combination counts were numerically higher than those in the antibiotic alone group (Okla et al., 2021; Albosolemy et al., 2022). This can occur due to luck, biased sample, or an unexpected interaction. Please note, the

manuscript did not put forth the claim that honey improves the efficacy of antibiotics. Conversely, the data are interpreted as demonstrating that honey alone (curative group) decreased bacterial counts in comparison with the negative control approaching but not exceeding that achieved by ceftriaxone. In this context, "supportive" means study group (honey administered with antibiotic), not a demonstrated synergistic effect.

This study provides a moderate but meaningful degree of novelty. Although the antimicrobial activity of honey against *P. aeruginosa* has been reported that the majority are in vitro or experimental topical applications to wounds (Syawalludin, 2019; Nasution et al., 2025). As a preliminary evidence, the present study provided in vivo data by using systemic infection model (intraperitoneal inoculation) and administration of certain local honey (*Apis dorsata* from South Sulawesi). Additionally, the comparison of preventive, curative and supportive activities under the same experimental set-up gives us preliminary evidence on time dependent effects of honey administration (Kozień et al., 2024). This study should be considered as preliminary preclinical evidence supporting more rigorous mechanistic experiments, than a definitive example of therapeutic efficacy (Mukherjee et al., 2022).

Several limitations are to be acknowledged in the interpretation of this study. Since this was a small design (n=6 per group), statistical power is low, and type II errors (or failing to detect real differences) are more likely to occur (Pleeging et al., 2020; Ogwu and Izah, 2025). This research did not state how randomization or blinding was done in the original manuscript, but the revised methods have rectified those omissions. The honey used was characterized for pH, sugar content, phenolics, flavonoids and hydrogen peroxide activity as well as sterility; however no batch-to-batch variability was evaluated (Li et al., 2023; Mahdi et al., 2024). The original design did not include sugar/osmolarity control; however, it was later amended to add an additional sugar control group. Only one dose of honey was tested and thus a dose-response analysis was not conducted. The route of administration (oral vs systemic) of honey for the prevention against IP infection is less clear and proposed systemic mechanisms (immunoregulation, antioxidant effects) were not directly quantified (Suresh et al., 2022; Kim et al., 2025). There were no measures on tissue bacterial burden (e.g., spleen, liver and peritoneal fluid), inflammatory biomarkers or histopathology; outcome measures were restricted to blood culture colony counts (Kasetty et al., 2021; Feng et al., 2022). Most of the recovered colonies were confirmed to be *P. aeruginosa* , based on Gram staining & oxidase testing, although in 4 samples this was not possible. Correction for multiple comparisons was performed (Bonferroni), this conservative correction increases the rate of false-negative, which may affect relevant results (Hardani et al., 2020). The Study conduct date (April 2025) and ethical approval date (October 2024) are both prospective as per the original manuscript but has been amended to fix a typed error. Lastly, without human safety, pharmacokinetic and pharmacodynamic data, the possible translational significance of these findings to human patients is restricted (Salam et al., 2023).

However, there are a few limitations to this study. This discovery, that *P. aeruginosa* colony counts reduced when forest honey was given orally in an in vivo mouse model, warrants further investigation into honey as a supplement adjunct for infection control (Adamu et al., 2021; Ratajczak et al., 2021). This is especially pertinent in light of the widespread increase in antibiotic resistance around the world, and an urgent need for alternative or adjunct therapies (Salam et al., 2023). Importantly, the rehabilitation for honey may be significantly efficacious only after infection is established (curative role), which should inform clinical trial design (Kozień et al., 2024). Several directions for future research need to be pursued. They are funded by larger samples with

enough statistically power. Mechanistic studies of oral honey use should involve direct measurements of immune parameters (e.g., cytokine profiles, phagocytosis assays), oxidative stress markers, and integrity of gut barriers following (Suresh et al., 2022; Kim et al., 2025). It would identify the best dosing regimen with dose-response studies. Comparative studies with medical-grade honey and other types of honey at the same concentrations as *Apis dorsata* would establish whether these effects are specific to *Apis dorsata* or generalizable (Mansur and Mukhtar, 2023). These should be considered generally as secondary outcomes alongside tissue bacterial burden and histopathology (Kasetty et al., 2021; Feng et al., 2022).

CONCLUSIONS

In this preliminary murine intraperitoneal infection model, oral administration of *Apis dorsata* forest honey was associated with lower blood-culture colony counts of *Pseudomonas aeruginosa* compared with untreated infected controls. The curative and supportive groups showed reduced bacterial counts, although ceftriaxone remained the most effective intervention. Because of the small sample size, limited outcome measures, lack of dose-response assessment, incomplete post-hoc statistical reporting, and absence of mechanistic data, these findings should be interpreted cautiously. Further studies with standardized honey characterization, rigorous randomization and blinding, expanded microbiological and immunological endpoints, and corrected statistical analysis are required before clinical relevance can be inferred.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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