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Insights into Hematological Parameters of Male Wistar Rats: Implications as Model Organisms for Urinary Tract Infection Studies with *Staphylococcus* sp, *Klebsiella* sp, EPEC, and *Bacillus* sp

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Abstract

Urinary tract infections (UTIs) are common in companion animals and are frequently used in experimental models to elucidate pathophysiology and evaluate interventions. Uropathogenic *Escherichia coli* (UPEC) is the standard inducer; however, culturing UPEC necessitates biosafety level-2 facilities, which restrict accessibility. This study investigated a viable alternative by evaluating whether other easily managed bacteria can induce a UTI phenotype in rats and alter basic hematological parameters. Twenty male Wistar rats were allocated to receive transurethral inoculation with *Staphylococcus* sp., *Klebsiella* sp., enteropathogenic *Escherichia coli* (EPEC), or *Bacillus* sp. (1×10^8 CFU/mL, once daily for three days); a saline group served as the negative control. After induction, whole blood was analyzed for total erythrocyte count, hemoglobin, and hematocrit. Erythrocytes were counted manually with a hemocytometer at $400\times$ magnification, hemoglobin was measured using the Sahli method, and hematocrit was read after microcentrifugation at 16,000 rpm for 5 minutes. Data were compared by one-way ANOVA ($\alpha=0.05$) followed by Tukey's test.

Across treatments, none of the bacterial challenges produced statistically significant changes versus saline ($p>0.05$). Descriptively, *Klebsiella* sp. yielded the highest average erythrocyte count ($4.24 \pm 1.71 \times 10^6$ cells/mm³) and hematocrit ($41.5 \pm 2.38\%$), whereas EPEC showed the lowest average hemoglobin (8.4 ± 0.43 g/dL). These findings suggest that, under the present conditions, transurethral inoculation with *Staphylococcus* sp., *Klebsiella* sp., EPEC, or *Bacillus* sp. does not measurably alter systemic hematological parameters in male Wistar rats. The protocol may therefore be insufficient to reproduce a robust UTI phenotype detectable by these indices alone. Future work should incorporate urine culture and urinalysis, histopathology of the urinary tract, inflammatory biomarkers, and refined dosing or timing to verify infection establishment. If confirmed, accessible organisms such as *Klebsiella* sp. could offer a feasible, lower-barrier model for UTI research where BSL-2 resources are constrained. Outcomes are presented as mean $\pm$ SD to aid interpretation and reproducibility here.

Keywords: Urinary Tract Infection (UTI), bacteria, erythrocytes, hemoglobin, hematocrit

A urinary tract infection (UTI) occurs when bacteria enter and proliferate within the urinary system, usually ascending from the urethra to the bladder and, in certain instances, to the kidneys. This process causes inflammation and can harm epithelial and deeper tissues along the tract (Flores-Mireles et al., 2015). UTIs are one of the most common infections that veterinarians see. According to Byron (2019), they affect about 3% to 19% of cats and about 14% of dogs. Upper urinary tract involvement (pyelonephritis) transpires when pathogens infiltrate the renal pelvis and parenchyma, resulting in bacterial proliferation that incites localized inflammatory responses and subsequent tissue damage. Aside from the immediate clinical signs and discomfort experienced by affected animals, UTIs can impair renal function, recur, and present significant diagnostic and therapeutic challenges for clinicians and pet owners.

Experimental rats are widely recognized as predictive models for studying urinary tract infections (UTIs) and for evaluating the efficacy and safety of pharmacological interventions (Frianto et al., 2015). For upper urinary tract infections (pyelonephritis), rats are particularly useful because their anatomical and physiological features mirror those of companion animals closely enough to enable cautious extrapolation. These characteristics permit systematic investigation of pathogenesis, disease progression, and therapeutic response, thereby advancing the development of UTI treatments in veterinary contexts.

Uropathogenic *Escherichia coli* (UPEC) is the standard inducer of UTIs in rat models. However, its cultivation and inoculation typically require biosafety level-2 (BSL-2) facilities, which can restrict reproducibility and broader adoption of such models (Pang et al., 2022). Consequently, alternative bacteria with infection dynamics comparable to UPEC warrant consideration. *Staphylococcus sp.*, *Klebsiella sp.*, Enteropathogenic *E. coli* (EPEC) (Indrawan, 2015), and *Bacillus sp.* (Bottone, 2010) are all implicated in UTIs and may serve as practical inducers in rats. Post-induction hematological profiling is essential to determine the extent to which these organisms recapitulate key UPEC-like features, given UPEC's central etiological role (Neal, 2008).

Bacterial infection perturbs systemic physiology, including blood composition. UTIs typically begin with urethral colonization and ascend to the bladder via adhesion factors, provoking lower tract inflammation that can extend to the kidneys. Inflammation may alter erythrocyte redistribution, while renal injury can disrupt erythropoietin regulation and downstream erythropoiesis in bone marrow (Mardana, 2015). Bacteremia, inflammatory signaling, and impaired erythropoietin regulation together can modify hematological profiles—particularly total erythrocyte count, hemoglobin, and hematocrit (Kohar et al., 2021).

Most rat UTI models focus on UPEC; rigorous, side-by-side evidence on whether non-UPEC bacteria can reproduce comparable clinical and hematological phenotypes remains limited, as do practical validation criteria for laboratories with constrained resources. This study designs and evaluates a male Wistar rat model of UTI using *Staphylococcus sp.*, *Klebsiella sp.*, EPEC, and *Bacillus sp.*, quantifying erythrocyte indices (erythrocyte count, hemoglobin, hematocrit) as surrogate markers of renal inflammation, and benchmarking against expected UPEC-associated patterns (Indrawan, 2015; Bottone, 2010; Neal, 2008). Developing UTI models operable outside BSL-2 while retaining translational relevance is timely to widen research access, accelerate therapeutic screening, and promote more rational antimicrobial stewardship (Frianto et al., 2015; Pang et al., 2022; Kohar et al., 2021).

MATERIALS AND METHODS

Animal preparation

A protocol for this study was approved by the Research Ethics Commission of Brawijaya University (No. 115-KEP UB-2022). Twenty clinically healthy, four-month-old male Wistar rats (*Rattus norvegicus*; 200–350 g) sourced from Singosari, Malang, were housed for 32 days (June–October) in Building B, Faculty of Veterinary Medicine, to allow full acclimatization. Animals were maintained in ventilated container-box cages covered with wire mesh, received pelleted feed twice daily, had ad libitum access to water, and were bedded on ~1-cm husk replaced daily; ambient temperature was controlled at 26–28 °C. This explicit husbandry reporting responds to a persistent gap—especially in tropical facilities—where acclimatization windows and environmental parameters are under-reported, limiting reproducibility and cross-study comparability. Our design introduces methodological novelty and urgency by (i) adopting

an intentionally extended acclimatization period to mitigate transport and novel-housing stress before experimentation, (ii) aligning with ARRIVE 2.0 recommendations for transparent reporting, and (iii) maintaining conditions near rats' thermal preferences to minimize cold-stress-related physiological noise—thereby improving welfare, data quality, and the ethical use of animals through better standardization (Percie du Sert et al., 2020; Kentner et al., 2021; Neville et al., 2023; Research Animal Resources, 2024; Velasco-González et al., 2025).

Bacteria preparation

We chose four common uropathogens—*Staphylococcus sp.*, *Klebsiella sp.*, *Bacillus sp.*, and *Escherichia coli*—to show the full range of urinary tract infections (UTIs) and to make it easier to compare them directly with uropathogenic *E. coli* (UPEC), which is the main cause of many UTIs (Yashir & Ariani, 2019). Despite comprehensive understanding of UPEC virulence, comparative evaluations among cocirculating species under standardized conditions are scarce—especially in veterinary settings—limiting external validity and evidence-based treatment. Our study fills this gap and provides originality by systematically comparing the pathogenic potential of these taxa through a cohesive experimental framework, enhancing interpretability and applicability for empirical treatment decisions (cf. Gajdács & Kárpáti, 2023; Rodrigues et al., 2024).

We counted the bacterial isolates by serial dilution and grew them on the right media: Nutrient Agar for *Bacillus sp.*, Trypticase Soy Agar for *E. coli*, MacConkey Agar for *Klebsiella sp.*, and other Enterobacterales. The working suspensions were standardized to approximately 1×10^8 CFU/mL, in accordance with established transurethral inoculation protocols in murine models (Luterbach & Mobley, 2018), and were stored at 2–8 °C for short-term preservation. This standardized workflow improves reproducibility and directly addresses the pressing demand for comparative data in light of increasing antimicrobial resistance in companion-animal UTI isolates (Rodrigues et al., 2024), while maintaining your original citations (Yashir & Ariani, 2019; Luterbach & Mobley, 2018).

Preparation of predicted UTI animal models

The generation of animal models of urinary tract infection (UTI) in rats was conducted at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Brawijaya University, on day 33, which was one day after the acclimatization period. The bacteria used for induction were transferred from the Microbiology Laboratory using a temperature-controlled box set at 8°C and subsequently stored in a refrigerator at the Clinical Pathology Laboratory, Brawijaya University. In the treatment group, bacteria were induced transurethrally. The control group, labeled as Group P1, was induced with physiological NaCl. Physiological NaCl, or normal saline (0.9% sodium chloride solution), is used as a control in experimental studies for several reasons. Firstly, it is isotonic, meaning it has the same salt concentration as the cells and blood of the rats, preventing any adverse effects on cell integrity or fluid balance. This ensures that any observed effects in the experimental group are due to the introduction of the test bacteria and not due to the solution used. Group P2 was induced with *Staphylococcus sp.* bacteria, while Group P3 received *Klebsiella sp.* bacteria induction. Enteropathogenic *Escherichia coli* (EPEC) bacteria-induced Group P4, and Group P5 received *Bacillus sp.* bacteria induction. Each rat was induced with 0.3 ml of bacteria at a concentration of 1×10^8 CFU/mL.

The transurethral route was chosen to mimic natural infection processes as in pet animals, where bacteria typically ascend the urethra to cause UTIs. Bacterial induction was carried out using a 26G IV catheter for three consecutive days. This strategy creates a more physiologically realistic model by recreating uropathogens' natural entrance and colonization routes, revealing insights into infection dynamics and host-pathogen interactions during natural infections.

The total erythrocyte counts

Rat blood, collected in an EDTA tube, was drawn with a standard erythrocyte pipette up to the 0.5 mark. Subsequently, Hayem's reagent was added by aspirating it up to the 101 mark. The blood mixed with the reagent was homogenized by sealing the ends of the pipette and gently moving it to create a figure-eight motion. To calculate the total erythrocyte count, a homogenized sample was then dropped into a

hemocytometer counting chamber and observed under a microscope at 400X magnification. Using a tally counter, the erythrocytes were counted within the marked grids of the hemocytometer.

Calculation of hemoglobin levels

Venous blood was collected into an EDTA tube. Using a Sahli pipette, 0.02 mL (20 µL) of whole blood was transferred into a Sahli hemoglobinometer tube prefilled with 0.1 N HCl to the “2” mark. The contents were gently mixed and allowed to stand for 1–2 minutes to permit complete conversion of hemoglobin to acid hematin. Distilled water was then added dropwise with gentle swirling until the solution’s color matched the standard on the Sahli comparator. The hemoglobin concentration (g/dL) was read directly from the graduated scale at the meniscus under consistent ambient light to reduce colorimetric bias.

Hematocrit determination (packed cell volume)

Hematocrit (Hct) was determined by the packed cell volume (PCV) method. Whole blood was drawn into heparinized microhematocrit capillaries to ~¾ of the tube length and sealed (clay/wax seal). Tubes were placed in a microhematocrit centrifuge (16,000 rpm, 5 min), with seals oriented outward, to achieve complete erythrocyte packing. Following centrifugation, the height of the red cell column and the total blood column (red cells + buffy coat + plasma) were measured using a standard microhematocrit reader. Hematocrit (%) was computed as:

$$\text{Hct (\%)} = \frac{\text{Height of packed red cells}}{\text{Total column height}} \times 100.$$

Centrifuge speed and timer functions were verified by routine calibration to ensure uniform separation. Each sample was run in duplicate; results differing by >1 percentage point prompted a repeat run. To ensure accuracy, PCV values were cross-validated against an automated hematology analyzer run on the same samples, and agreement was monitored with Levey–Jennings charts and Westgard rules; any systematic bias triggered instrument checks and recalibration. Measurements were read at eye level, under consistent illumination, avoiding inclusion of the buffy coat in the red cell height.

Data analysis

Quantitative data for total erythrocyte count, hemoglobin concentration, and hematocrit were analyzed in IBM SPSS Statistics 23® using one-way analysis of variance (ANOVA). Prior to the omnibus test, assumptions were evaluated: normality was assessed for each group ($p > 0.05$), indicating approximately normal distributions, and homogeneity of variances was confirmed ($p > 0.05$). Group means were compared with a two-sided $\alpha = 0.05$; thus, ANOVA results were considered statistically significant at $p < 0.05$. When the omnibus ANOVA indicated a significant effect, pairwise differences were examined using Tukey’s honestly significant difference (HSD) post hoc test to control the family-wise error rate, with adjusted p-values < 0.05 interpreted as significant. Where relevant, results should be reported with F-statistics, degrees of freedom, and exact p-values, and may be complemented by effect sizes (e.g., η^2) and 95% confidence intervals to aid interpretation.

RESULTS AND DISCUSSION

Effect of induction of *Staphylococcus sp.*, *Klebsiella sp.*, EPEC, and *Bacillus sp.* on total erythrocytes of male Wistar rats

Erythrocytes are one of the solid parts that make up blood. Erythrocytes are in charge of moving oxygen around the body so that it can be used for metabolic needs. The total erythrocyte count can offer insights into a person's health status. The analysis of total erythrocyte data indicated that the induction of *Staphylococcus sp.*, *Klebsiella sp.*, EPEC, and *Bacillus sp.* in male Wistar strain white rats (*Rattus norvegicus*) resulted in no significant differences ($p > 0.05$) when compared to the negative control group, as illustrated in Fig 1.

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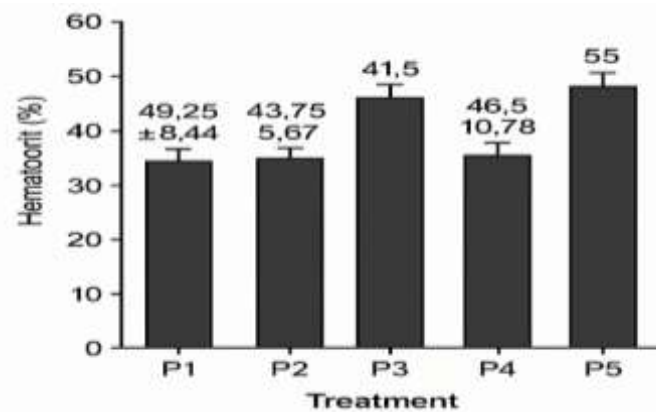


Figure 1. Average total erythrocytes of male Wistar rats after treatment

Information:

Different notations indicate significant differences between treatment groups ($p < 0.05$). P1: NaCl induction, P2: *Staphylococcus sp.* P3: Induction of *Klebsiella sp.* P4 Induction of EPEC P5: Induction of *Bacillus sp.*

A downward shift in average total erythrocyte counts across all bacterially induced groups (P2, P3, P4, P5) relative to the negative control (P1, physiological NaCl). This pattern is biologically plausible: bacterial infection can both lyse circulating erythrocytes and impair erythropoiesis, producing net red cell loss. In the *Klebsiella*-challenged animals, the mean erythrocyte count was $4.24 \pm 1.71 \times 10^6$ cells/mm³. Although this value did not differ significantly from P1 or the other treatment groups ($p > 0.05$), it lies below the commonly cited reference interval for rats ($6.76\text{--}9.20 \times 10^6$ cells/mm³) (Wahdaningsih et al., 2020). Group P3 also showed a lower mean than P1 and was below the reference range, indicating a trend toward anemia even where pairwise statistical significance was not observed.

The mechanistic basis for these hematologic changes is consistent with pathogen-specific virulence. *Klebsiella spp.* produce siderophores such as aerobactin and colibactin that sequester host iron, predisposing to erythrocyte lysis and limiting iron availability for erythropoiesis (Khaertynov et al., 2018). In parallel, lipopolysaccharide (LPS)–driven inflammation activates IL-6 and TNF- α , promoting macrophage responses and hepcidin-mediated hypoferremia, which further restricts iron for red cell production (Ganz, 2009). Ascending infection can progress to pyelonephritis, disrupting renal erythropoietin (EPO) regulation and suppressing marrow erythropoiesis (Mardana, 2015). In the EPEC-induced group (P4), the mean erythrocyte count was $4.91 \pm 1.98 \times 10^6$ cells/mm³; despite no significant differences versus P1, P2, P3, or P5 ($p > 0.05$), values were below the reference interval (Wahdaningsih et al., 2020). EPEC adhesion (fimbriae, intimin) and virulence determinants (type IV bundle-forming pili, LEE) elicit local injury and robust innate signaling (e.g., LPS, flagellin), amplifying TNF- α –driven inflammation and iron sequestration (Kaur et al., 2023; Ganz, 2009). Collectively, erythrocyte lysis plus reduced erythropoiesis—via iron restriction and potential EPO suppression—offer a coherent explanation for the observed reductions in total erythrocyte counts across infected groups.

The animals in the *Bacillus sp.*-induced group (P5) had an average of $5.18 \pm 2.85 \times 10^6$ cells/mm³, which was lower than the total erythrocytes in the P1 group, which served as the control. The average total erythrocyte findings did not differ substantially ($p > 0.05$) from groups P1, P2, P3, and P4, which served as the negative control. Wahdaningsih et al. (2020) reported that the range of total normal erythrocytes in white rat is $6.76\text{--}9.2 \times 10^6$ cells/mm³. The virulence and adhesion factors of *Bacillus sp.* were responsible for the reduction in total erythrocytes. By causing damage to hemolysin BL (HBL)-affected tissue, bacteria adhere to host tissue and spread deeper into it. Because germs can enter the bloodstream through damaged tissue capillaries, hemolysin BL (HBL) can also cause erythrocyte lysis. The overall number of erythrocytes in the blood decreases as a result of erythrocyte lysis in circulation.

The mean total erythrocyte count in the *Staphylococcus sp.*–exposed group (P2) was $6.10 \pm 1.98 \times 10^6$ cells/mm³. Statistically, P2 did not differ from any comparison group (P1, P3, P4, or P5; all $p > 0.05$), including the negative control (P1). Nonetheless, the P2 mean was numerically lower than P1 and fell below

the published physiological range for Wistar rats ($6.76\text{--}9.20 \times 10^6$ cells/mm³), indicating a potential downward shift in erythrocyte status despite the absence of a significant between-group effect (Wahdaningsih et al., 2020).

Biologically, a reduction in erythrocytes following *Staphylococcus* sp. exposure is plausible and coherent with known pathophysiology. *Staphylococcus* sp virulence factors—such as proteases, coagulase, and α -toxin (α -haemolysin)—can drive local tissue injury and hemolysis, directly decreasing circulating red cells (Dewi, 2016). Concurrently, infection triggers pro-inflammatory cytokines (e.g., IL-1, TNF- α), which promote iron sequestration and hypoferremia to support phagocytic antimicrobial activity; this iron-restriction response, in turn, hampers erythropoiesis (Ganz, 2009). Taken together, erythrocyte loss from toxin-mediated lysis, coupled with impaired red cell production under inflammation-induced iron limitation, offers a mechanistic explanation for the lower mean observed in P2 relative to the negative control, even though group differences did not reach statistical significance.

Effect of induction of *Staphylococcus* sp., *Klebsiella* sp., EPEC, and *Bacillus* sp. on hemoglobin levels of male Wistar rats

Hemoglobin is a protein in red blood cells that helps carry carbon dioxide from body tissues back to the lungs to be breathed out and binds oxygen from the lungs to the rest of the body. Laboratories often check the amount of hemoglobin in the blood to see how well the body moves oxygen around. The analysis of hemoglobin level data indicated that the male Wistar strain and the induction of *Klebsiella* sp. in white rats (*Rattus norvegicus*) yielded significantly different results ($p < 0.05$) in comparison to the negative control group. Figure 2 illustrates that the induction of *Staphylococcus* sp., EPEC, and *Bacillus* sp. in rats did not exhibit significant differences ($p > 0.05$) when compared to the control group.

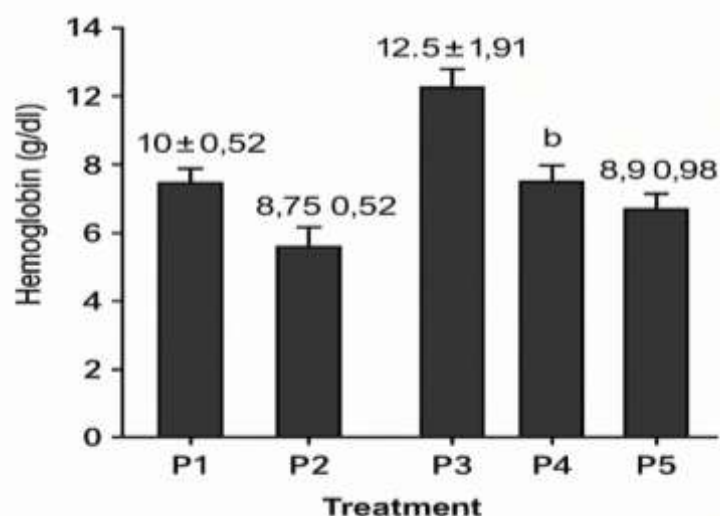


Figure 2. Average hemoglobin levels of male Wistar rats after treatment

Information:

Different notations indicate significant differences between treatment groups ($p < 0.05$). P1: NaCl induction, P2: *Staphylococcus* sp. P3: Induction of *Klebsiella* sp. P4 Induction of EPEC P5: Induction of *Bacillus* sp

Here we report group-wise differences in hemoglobin (Hb) consistent with both pathogen effects and physiological responses. Compared with the negative control (P1), mean Hb was lower in the **Staphylococcus** sp.-infected group (P2: 8.75 ± 0.52 g/dL) and lowest in the EPEC group (P4: 8.40 ± 0.43 g/dL); values in P5 were also reduced relative to P1. By contrast, Hb in P3 was higher than in P1. One-way ANOVA followed by Tukey's test indicated no significant differences among P1, P2, P4, and P5 ($p > 0.05$), but P3 differed significantly from P4 and P2 ($p < 0.05$). Notably, the mean Hb values observed in P2 and P4 fall below the reported reference range for Wistar rats ($\approx 11.5\text{--}16.1$ g/dL), suggesting clinically meaningful reductions in oxygen-carrying capacity (Wahdaningsih et al., 2020).

The pattern is biologically plausible. In the P2 (*Staphylococcus sp.*) cohort, Hb reductions are consistent with hemolysis and inflammation driven by bacterial virulence. *Staphylococcus sp.* produces exotoxins such as α -toxin (α -haemolysin) that damage endothelial and epithelial cells; toxin-mediated membrane injury can lyse erythrocytes and release intracellular hemoglobin, thereby lowering measured circulating Hb (Dewi, 2016). Concurrently, infection-related cytokine responses activate macrophages and heighten iron demand within inflamed tissues; along with direct toxin effects, this milieu can exacerbate erythrocyte turnover and reduce circulating Hb. The EPEC (P4) group showed the largest decrement, aligning with robust mucosal and systemic inflammatory responses that may intensify hemolysis and hemodilution. In contrast, elevated Hb in P3 may reflect hemoconcentration (e.g., dehydration) or a transient erythropoietic response to relative hypoxia; however, these interpretations should be made cautiously and verified against hydration status and reticulocyte indices. Taken together, the data indicate pathogen-specific impacts on Hb, with *Staphylococcus sp.* and EPEC associated with the greatest decreases, and underscore the importance of integrating clinical context (hydration, inflammation markers) when interpreting hematologic endpoints (Wahdaningsih et al., 2020; Dewi, 2016).

The *Bacillus sp.*-induced group (P5) showed a mean hemoglobin concentration of 8.9 ± 0.98 g/dL. This value did not differ significantly from P1, P2, or P4 ($p > 0.05$) but was significantly lower than P3 ($p < 0.05$). Relative to the negative control (P1), P5 exhibited a decrease in hemoglobin, falling below the reference range reported for white rats (11.5–16.1 g/dL) (Wahdaningsih et al., 2020). The observed reduction is biologically plausible given known *Bacillus* virulence features—capsule, S-layer, peptidoglycan architecture, and particularly the hemolysin BL complex—which facilitate adhesion, tissue injury, and hemolysis. Local inflammation with cytokine activation promotes macrophage-mediated clearance of damaged erythrocytes, while hemolysin-mediated membrane disruption accelerates red-cell lysis, releasing hemoglobin into the extracellular milieu and thereby lowering measured circulating levels (Dewi, 2016).

By contrast, the *Klebsiella sp.*-induced group (P3) had a mean hemoglobin concentration of 12.5 ± 1.91 g/dL, which differed significantly from P2, P4, and P5 and also from the negative control P1 (all $p < 0.05$). Although elevated relative to P1, the P3 mean remained within the reported normal range for white rats (11.5–16.1 g/dL) (Wahdaningsih et al., 2020). The increase may reflect physiological variation (e.g., transient hemoconcentration or stress-related shifts) under experimental conditions. An explanation based on aplastic anemia is unlikely, as aplastic anemia is classically characterized by reduced marrow output and lower hemoglobin, not higher (Beutler & Waalen, 2006). Taken together, these findings suggest that *Bacillus sp.* exposure is associated with hemolysis-linked declines in hemoglobin, whereas *Klebsiella sp.* exposure—under the present protocol—did not depress hemoglobin and may even produce modest elevations within the physiological range. Further work incorporating reticulocyte indices and markers of hemolysis (e.g., haptoglobin, indirect bilirubin) would strengthen mechanistic inference while controlling for hydration status and other confounders.

Effect of Induction of *Staphylococcus sp.*, *Klebsiella sp.*, EPEC, and *Bacillus sp.* on the Hematocrit Value of Male Wistar Rats

Hematocrit is the ratio of the number of blood cells, such as red blood cells, to the total volume of blood. Hematocrit is shown as a percentage. The hematocrit value is closely linked to the body's erythrocytes. This is based on data analysis of induced hematocrit values for *Staphylococcus sp.*, *Klebsiella sp.*, EPEC, and *Bacillus sp.* The male Wistar strain of white rats (*Rattus norvegicus*) exhibited results that were not significantly different ($p > 0.05$) from the negative control group, as illustrated in Figure 3.

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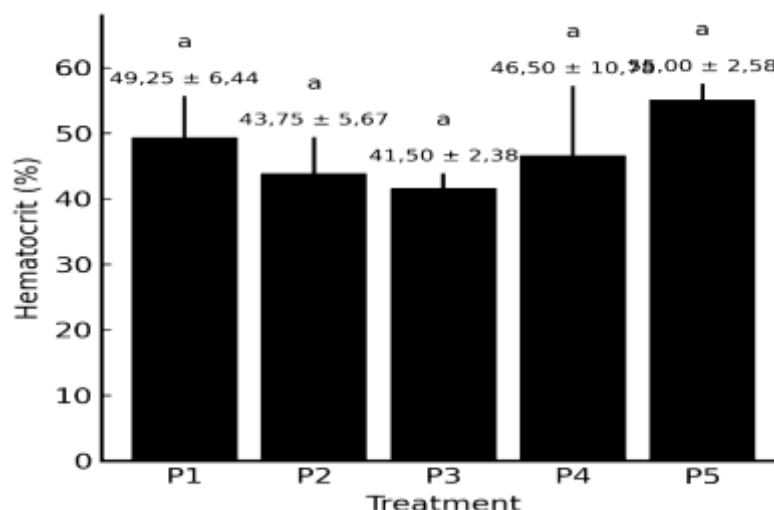


Figure 3. Average hematocrit value of male Wistar rats after treatment

Information:

Different notations indicate significant differences between treatment groups ($p < 0.05$). P1: NaCl induction, P2: *Staphylococcus sp.* P3: Induction of *Klebsiella sp.* P4 Induction of EPEC P5: Induction of *Bacillus sp.*

Relative to the negative control (P1; physiological NaCl), hematocrit (PCV) values decreased in the bacteria-induced groups P2 (*Staphylococcus sp.*), P3 (*Klebsiella sp.*), and P4 (EPEC), consistent with the principle that reduced erythrocyte count lowers hematocrit (Dewi, 2016). Across groups, the observed means remained within the physiological range reported for white rats (37.2–50.6%; Wahdaningsih et al., 2020), indicating no overt anemia or polycythemia at the group level.

In the *Klebsiella sp.*-induced group (P3), the mean hematocrit was $41.5 \pm 2.38\%$, and in the *Staphylococcus sp.*-induced group (P2) it was $43.75 \pm 5.67\%$. When compared with P1 and with the other treatment groups (P2, P4, P5), these differences were not statistically significant ($p > 0.05$). Although both P3 and P2 showed descriptive reductions versus P1, values remained within the reference interval (Wahdaningsih et al., 2020). Biologically, these patterns are plausible: virulence factors—such as siderophores and lipopolysaccharides (LPS) in *Klebsiella sp.*—may perturb erythrocyte integrity or erythropoiesis, lowering the packed cell volume; similarly, *Staphylococcus sp.* can reduce circulating erythrocyte numbers and thereby decrease hematocrit (Dewi, 2016).

For the EPEC-challenged group (P4), the mean hematocrit was $46.5 \pm 10.78\%$, with no significant differences versus P1 or other groups ($p > 0.05$). Although descriptively lower than P1 in the dataset, P4 values also fell within the normal range for white rats (Wahdaningsih et al., 2020). In contrast, the P5 group exhibited a higher mean hematocrit than P1 (no p -value provided), a pattern consistent with hemoconcentration arising from reduced body fluids; dehydration elevates the proportion of erythrocyte volume relative to total blood volume and thus increases the hematocrit (Rumayar et al., 2016).

In the *Bacillus sp.*-induced group (P5), the mean hematocrit was $55.0 \pm 2.58\%$. Although this value was higher than typical reference ranges, group comparisons showed that mean hemoglobin in P5 did not differ significantly from P1–P4 ($p > 0.05$). Notably, the P5 mean hemoglobin trended higher than the negative control. The elevated hematocrit in P5 is plausibly consistent with relative hemoconcentration, which can arise when plasma volume decreases (e.g., reduced fluid intake, fever, or polyuria during infection).

Across all bacterial challenges, induction of the UTI model was generally associated with reductions in hematocrit, hemoglobin, and total erythrocyte count relative to controls. The smallest mean decline in total erythrocytes and hematocrit occurred in the *Klebsiella sp.*-induced group, whereas the smallest mean decrease in hemoglobin was observed in the EPEC-induced group. Importantly, *Klebsiella sp.* induction did not produce an anemic profile: average hemoglobin and hematocrit remained within normal limits, although the average erythrocyte count was slightly below the customary threshold. A similar pattern was seen with the other bacterial groups; while modest shifts occurred, mean

hemoglobin and hematocrit generally stayed within acceptable ranges and did not meet criteria for anemia.

These findings suggest species-specific hematological responses to UTI induction, with *Bacillus* sp. associated with higher hematocrit and *Klebsiella* sp. and EPEC showing comparatively attenuated declines in red-cell indices. From a physiological perspective, such patterns may reflect differing balances between inflammation-driven erythrocyte dynamics and fluid balance alterations during acute infection.

Finally, our induced UTI model reproduces the natural ascending route of infection by transurethral inoculation, providing a controlled platform to compare pathogens. Nevertheless, key differences from spontaneous disease remain—most notably the standardized inoculum, controlled host and environmental variables, and the compressed timeline of clinical progression. These distinctions should be considered when extrapolating to natural infections, and they underscore the need for complementary studies that incorporate variable inocula, host susceptibility factors, and longitudinal follow-up.

CONCLUSION

One-way ANOVA showed no significant group effect for total erythrocyte count or hematocrit (both $p > 0.05$). Across treatments, erythrocyte counts remained stable, with the *Klebsiella* sp. group exhibiting the smallest numerical decline. Hematocrit values likewise did not differ significantly among groups ($p > 0.05$), and the *Klebsiella* sp. group again showed the lowest apparent reduction. In contrast, hemoglobin concentration demonstrated a significant difference ($p < 0.05$), driven by a lower mean in the *Klebsiella* sp. group relative to the negative control on post-hoc testing. Notably, the EPEC group displayed the least decline in hemoglobin.

Taken together, these findings indicate that the current induction conditions produced limited hematological perturbations, with only a modest, bacteria-specific effect observed for hemoglobin. This pattern suggests that the present model may primarily influence parameters outside of basic red cell indices or that the exposure intensity/duration was insufficient to elicit broader hematologic change. Future work should refine the induction protocol (e.g., inoculum dose, exposure window, or combined host stressors), incorporate additional readouts (e.g., reticulocyte indices, inflammatory markers, iron metabolism parameters), and extend sampling to multiple time points to capture transient effects. Such enhancements will help clarify pathogen-specific hematologic signatures, strengthen biological interpretation, and improve the translational value of this model for advancing UTI diagnostics and therapeutic strategies in veterinary contexts.

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Conflict Of Interest:

The authors declare that there is no conflict interest in this manuscript.

Authors Contributions

Main conceptual and Experimental Design: Tiara Widyaputri and Yudit Oktanella collaborated on designing the experimental protocols, outlining the procedures for inducing urinary tract infections in male Wistar rats using the specified bacterial strains. Manuscript Preparation - Drafting: Ilham Adi Pangestu and Dyah Ayu Oktavianie A Pratama collaborated on drafting the initial manuscript, synthesizing the experimental findings into a cohesive narrative that highlights the hematological response of Wistar rats to urinary tract infections induced by different bacterial pathogens. Manuscript Revision and Editing: all authors jointly revised and edited the manuscript based on feedback from peer reviewers and advisors,

refining the language, structure, and organization of the paper to enhance its readability and scholarly impact

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