



EVALUATING ANTIMICROBIAL PEPTIDE RESISTANCE AND HOST IMMUNE RESPONSES IN STAPHYLOCOCCUS AUREUS INFECTIONS

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Abstract

Understanding the adaptation of *Staphylococcus aureus* and host immune response during infection is an important challenge with regard to the development of Antimicrobial peptide (AMP) resistance. This study tested the correlation between antimicrobial peptide resistance, immune gene expression, bacterial persistence and host survival using experimental data from a *Tenebrio molitor* insect infection model. Experimentally generated data (2,100 records) consisting of bacterial-load measurements, survival observations, and quantitative polymerase chain reaction data were subjected to quantitative secondary-data analysis. Nine strains of *S. aureus*, including the ancestor, the AMP-sensitive and the AMP-resistant variants were investigated under both control and immune-gene knockdown conditions. Bacterial load was determined by colony-forming unit counts and host immune responses by the expression of tenecin 1, tenecin 2, tenecin 4, and prophenoloxidase genes. Results showed that higher median bacterial loads were found after AMP knockdown on Day 7 (720 versus 328 CFU/100 μ L). AMP knockdown was associated with a 9.0-percentage-point increase in mortality, whereas prophenoloxidase knockdown was associated with a 14.7-percentage-point increase. Immune gene expression was reduced and there were heterogeneous strain-specific survival patterns, indicating complex interactions between bacterial resistance and host defenses. Resistance classes were not however always correlated with the severity of infections. These results highlight the need for innate immune mechanisms in controlling bacterial infection and offer clues to the host-pathogen relationship associated with resistance, paving the way for further study on bacterial antimicrobial resistance and immune regulation.

Keywords: *Staphylococcus aureus*; Antimicrobial Peptide Resistance; Host Immune Response; Bacterial Load; *Tenebrio molitor*.

1. Introduction

Staphylococcus aureus is a clinically important Gram-positive bacterium that causes a wide spectrum of infections from superficial skin and soft tissue infections to more serious systemic infections such as bacteremia, pneumonia, endocarditis, and sepsis. It has numerous virulence factors, a range of adaptive capabilities to host environments and mechanisms that promote immune system evasion. The development of antimicrobial resistance (AMR) has made infection management more complex and raised the importance of understanding how bacteria survive when subjected to host immune pressure. To evade innate immune recognition, the bacterium uses multiple mechanisms to interfere with immune signaling and persist in infected tissues (De Jong et al., 2019). Host adaptation also influences the physiology and virulence factors of bacteria, which can enhance their survival (Howden et al., 2023). Therefore, it is necessary to investigate bacterial resistance in addition to host immune responses to understand the processes of infection and to find new strategies for the treatment of infections other than antibiotics (Douglas et al., 2023).

Antimicrobial peptides (AMPs) are components of innate immunity which are crucial for the inhibition of bacterial growth by disrupting the bacterial membrane and other mechanisms. *S. aureus* can, however, develop resistance by changing the composition of its cell envelopes, their surface charge, and the way in which they regulate stress response. Experimental studies have shown that resistance to AMPs can change the pharmacodynamic parameters of the bacteria other than the standard MIC-based parameter (El Shazely et al., 2020). Insect immune environments also affect the survival traits of AMP-resistant bacterial populations (El Shazely et al., 2019). Resistance mechanisms can include complex genetic changes that make bacteria resistant to a variety of antimicrobial compounds (Maron et al., 2025). The effect of combining antimicrobial peptides has been studied to limit the emergence of resistance (Maron et al., 2022). Thus, knowledge of the mechanisms of antimicrobial action on the membrane and bacterial adaptation is key to the development of effective interventions (Ganesan et al., 2023; Tajer et al., 2024).

The outcome of bacterial clearance, severity of infection and survival depend on host immune response. The components of innate immunity include antimicrobial peptides, cellular defense, inflammatory signaling, and pathogen-recognition pathways. Bacterial resistance properties and host immunity can potentially interact to affect infection outcomes but differ across experimental systems (Jahan et al., 2022). Immunometabolic regulation is involved in host defense and bacterial persistence during *S. aureus* infection (Horn & Kielian, 2021). Moreover, virulence factors such as protein A hinder immune recognition and protective responses against bacteria (Bear et al., 2023). Another host defense mechanism against *S. aureus* is antibody-mediated protection (Chen & Missiakas, 2024). In addition, antimicrobial peptides are being explored for their potential therapeutic use against resistant bacterial pathogens (Mba & Nweze, 2022).

While antimicrobial resistance and host immunity have been explored in great detail, it remains important to study the interaction between the two in relation to bacterial burden, immune gene expression and host survival. Experimental observations indicate that there may be compromises in the resistance of bacteria to other components of a host's immune system that accompany their resistance to AMPs (El Shazely & Rolff, 2025). These relationships, however, necessitate a combined assessment of resistance phenotypes, immune suppression and infection outcomes. Prior studies also reinforce the intricacy of bacterial resistance mechanisms, pointing out the necessity to investigate resistance in a suitable biological context instead of just laboratory susceptibility readings (Tajer et al., 2024). Experimental infection data can thus be integrated to explain the relationships between AMP resistance, immune response of the host, persistence of bacteria and survival.

In the present work, antimicrobial peptide resistance and host immune responses against *S. aureus* infection were assessed using experimental data from an insect host model. Specifically, the study aims to compare the burden and severity of infection when comparing antimicrobial peptide-resistant versus antimicrobial peptide-sensitive *S. aureus*, to quantify host immune responses using measurement of gene expression of antimicrobial peptides and prophenoloxidase, to determine the impact of immune gene knockdown on the bacterial burden and/or host survival, and to investigate the relationship between antimicrobial peptide resistance, host immune responses, and bacterial infection outcomes. In this way, the study give an integrated view of bacterial adaptation, host defense mechanisms, and infection progression in the experimental infection model.

2. Materials and Methods

2.1. Research Design and Dataset Description

The present study was designed with a quantitative secondary data analysis to assess antimicrobial peptide (AMP) resistance and host immune response during *Staphylococcus aureus* infection. The dataset consisted of 7 experimental CSV files with 2,100 observations in total (540 with bacterial-load data, 1,200 for survival data, and 360 for quantitative PCR (qPCR) data). Nine variants of *S. aureus*-ancestral, AMP-sensitive and AMP-resistant were used in the experiments with the female *T. molitor* beetles. An analysis was made of the resistance to bacteria, the expression of immune genes, the amount of bacteria and the survival of the host after immune-gene knockdown (Rolff & El Shazely, 2022).

2.2. Data Preprocessing and Experimental Grouping

The data sets were checked for missing values, inconsistent labels, experimental classification, and experimental measurement format. The quantitative analysis was performed without the inclusion of one bacterial-load measurement. The bacterial strains were classified as either AMP-sensitive or AMP-resistant based on their known acquisition histories, and evolutionary line membership was considered when comparing strains. Bacterial strain, control or knockdown treatment and sampling day were used to categorize experimental groups. Ambiguous treatment labels and lack of group identifiers from the qPCR records required verification before analysis.

2.3. Assessment of Antimicrobial Peptide Resistance

The documented resistance classification of *S. aureus* strains was used to assess resistance to AMP. Resistant variants were compared to AMP-sensitive controls of the same evolutionary lines. Bacterial load and survival were assessed together with recorded resistance-associated mutation categories (rpo, pmtR/S, and nsrR/S). The differences in resistance were analyzed in the absence and presence of immune knockdown. From the available data, antimicrobial susceptibility testing was not performed due to the absence of minimum inhibitory concentration data

2.4. Assessment of Host Immune Responses

Host immune responses were evaluated using quantitative real-time PCR (qPCR) measurements of four immune-related target genes: tenecin 1 (T1), tenecin 2 (T2), tenecin 4 (T4), and prophenoloxidase (PO), with RPL27 used as the reference gene for normalization. The gene-expression measurements obtained on Day 1 and Day 7 were compared among the various bacterial strains and experimental treatments. The ΔC_t method was used to normalize expression values to that of the reference gene RPL27. Comparisons emphasized transcription changes of genes involved in bacterial resistance and knockdown of immune genes.

2.5. Bacterial Load and Host Survival Analysis

The bacterial load was determined by measuring the number of colony-forming units (CFUs) from 100 μ L of beetle hemolymph on Day 1 and Day 7 after infection. Comparisons were made with differences between bacterial strains and with control and combined T1, T2 and T4 knockdown treatments. Host survival was compared with both the AMP-knockdown and PO-knockdown data sets and follow-up extended to 30 days. To explore differences in infection outcome between experimental groups, survival time and mortality indicators were used. Survival analyses included right-censored observations

2.6. Statistical Analysis

Descriptive statistical analyses were performed to summarize bacterial-load measurements, immune-gene expression, and host-survival outcomes. Bacterial burden was summarized using medians and mean $\log_{10}(\text{CFU} + 1)$ values, while immune-gene expression was evaluated using normalized ΔC_t values and relative expression estimates. Mortality percentages were calculated for experimental groups, and Kaplan–Meier survival curves were used to describe survival probabilities over 30 days. Comparisons were conducted across bacterial strains, resistance categories, sampling days, and immune-knockdown treatments. Because formal inferential analyses were not reported, all observed differences were interpreted descriptively without claims of statistical significance. Associations between bacterial burden and mortality were evaluated at the experimental-group level.

3. Results

3.1. Dataset Characteristics and Experimental Group Distribution

The experimental data included 540 observations of bacterial load, 1200 observations of the number of bacteria that survived, and 360 observations of bacterial DNA by qPCR. Nine strains of *S. aureus* were investigated: one original strain and three strains used as controls for AMP sensitivity and five strains that were resistant to AMP. There were two measurements of the bacterial load, one at day 1 and one at day 7, and survival was tracked for 30 days. There were no data available on bacterial load in one case, giving 539 valid samples of bacterial load. Sham-injected controls were also included in the survival datasets. The data outlined in Table 1 are summarized and the number of measurements available allows complementary analysis of bacterial persistence, immune transcription, and survival. Individual records from different experiments were not considered to be from the same beetles.

Table 1. Characteristics and Distribution of Experimental Datasets

Experimental dataset	Records	Main measurements	Observation period
AMP knockdown – bacterial load	540	CFU/100 μ L	Days 1 and 7
AMP knockdown – survival	600	Survival time, mortality	30 days
PO knockdown – survival	600	Survival time, mortality	30 days
AMP qPCR – Day 1	90	T1, T2, T4, RPL27	Day 1
AMP qPCR – Day 7	90	T1, T2, T4, RPL27	Day 7
PO qPCR – Day 1	90	PO, RPL27	Day 1
PO qPCR – Day 7	90	PO, RPL27	Day 7

3.2. Antimicrobial Peptide Resistance Patterns Across *S. aureus* Strains

The bacterial strains were classified based on their known resistance to antimicrobial peptides. Tenecin 1 resistance-associated classification and tenecin 1 and tenecin 2 resistance-associated classification were observed in five variants. There was a wide range of host mortality in different strains. For instance, in the AMP-knockdown survival experiment, T1-1L caused 76.7% mortality in control hosts while only 10.0% mortality in AMP-knockdown hosts. In contrast, the

sensitive strain C1 had a mortality of 20.0% and 80.0% when treated with the same. These differences suggested that the outcome of infection was strain- and host-immune-dependent. Figure 1 shows survivability by strain for the two survival experiments, and it is clear that the infection outcomes are significantly different between the strains. Mortality values should not be regarded as direct measures of antimicrobial susceptibility and the resistance categories were experimentally documented classifications.

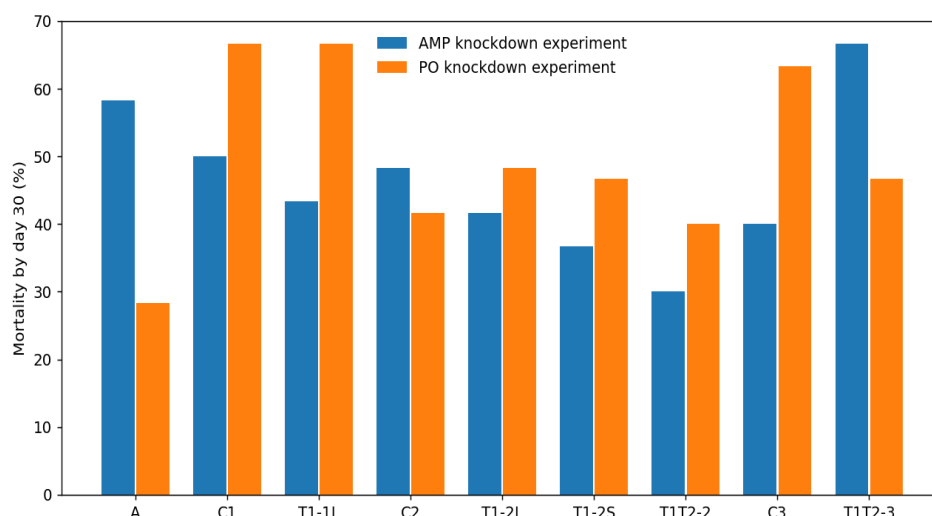


Figure 1. Strain-Specific Host Mortality in AMP- and PO-Knockdown Experiments

3.3. Effects of AMP Knockdown on Bacterial Load

The bacterial-load data indicated that there were temporal differences between the control and AMP-knockdown treatments. The median bacterial loads for control and AMP knockdown were 4,500 and 4,100 CFU/100 μ L, respectively, on Day 1. The median bacterial loads in both groups were lower by Day 7, but still higher in AMP-knockdown beetles (720 CFU/100 μ L) compared to controls (328 CFU/100 μ L). Overall, these results suggest a descriptive relationship between lower expression of AMP and increased bacterial persistence at the later sampling time. As shown in Table 2, the number of bacteria decreased after the first 7 days, and the descriptive drop in the bacterial burden was less pronounced in the AMP-knockdown beetles. These pooled values, however, do not indicate statistically significant effects when adjusted for evolutionary lineage and strain.

Table 2. Bacterial Load Under Control and AMP-Knockdown Conditions

Sampling day	Treatment	Valid n	Median CFU/100 μ L	Mean $\log_{10}(\text{CFU} + 1)$
Day 1	Control	135	4,500	3.54
Day 1	AMP knockdown	134	4,100	3.56
Day 7	Control	135	328	2.44
Day 7	AMP knockdown	135	720	2.88

3.4. Host Immune Gene Expression Responses

A different expression of tenecin was seen between control and AMP-knockdown treatments by normalized qPCR measurements. On Day 1, mean Δ Ct values for T1, T2, and T4 were 3.85, -4.40, and -4.21 in controls compared with 9.02, -2.01, and -1.77 following knockdown. These differences were also seen on Day 7, which is in line with decreased expression of the target genes in the knockdown groups. The estimated relative expression levels of each of the 3 genes across the 2 collection days using pooled control-group means as calibrators ranged from ~0.014 to ~0.191. As shown in Figure 2, there were consistently higher Δ Ct values after AMP knockdown, which means there were fewer transcripts found compared to the normalised amount. The Day 1 qPCR group assignments were re-created from the order of the samples listed in the README and have yet to be verified against the original laboratory records. The Day 1 AMP qPCR group assignments were reconstructed from the sample-order information provided in the accompanying documentation; therefore, these assignments remain provisional until verified against the original experimental records.

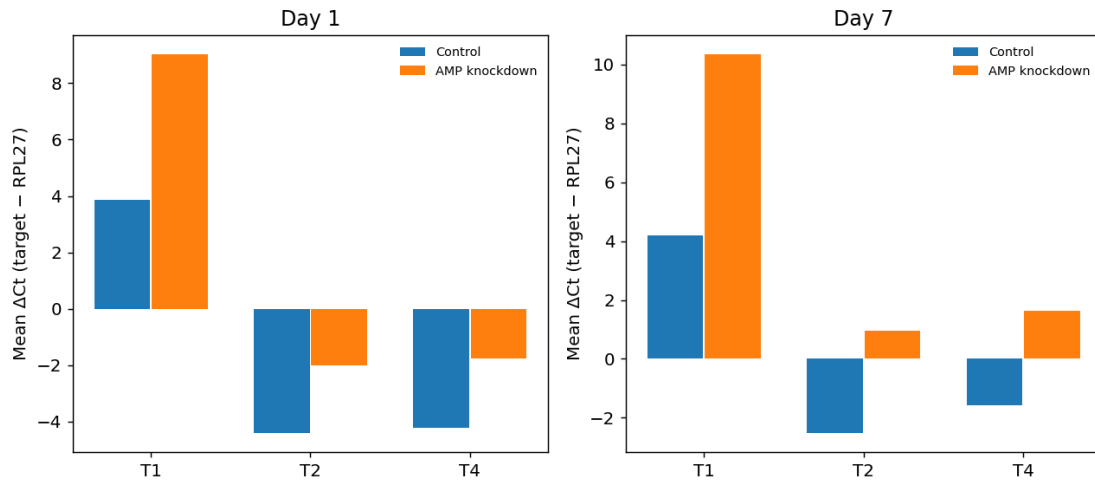


Figure 2. Antimicrobial Peptide Gene Expression Across Experimental Treatments

3.5. Prophenoloxidase-Mediated Immune Responses

The expression of prophenoloxidase was assessed separately from 180 qPCR observations taken on Days 1 and 7. The mean ΔC_t values for the knockdown group were higher than those of the controls at both time points, signifying decreased expression of the PO transcript relative to the control. The relative expression levels estimated were 0.245 (about 75.5% lower) and 0.114 (about 88.6% lower) on Days 1 and 7, respectively, compared with the respective pooled controls. Table 3 shows that the expression of PO transcripts is decreased after knockdown, especially at Day 7. The results presented here agree with the idea of transcriptional repression of the immune pathway targeted; but they don't actually measure the activity of PO enzymes.

Table 3. Prophenoloxidase Gene Expression in Control and Knockdown Groups

Sampling day	Treatment	n	Mean ΔC_t	SD
Day 1	Control	45	11.63	1.32
Day 1	PO knockdown	45	13.66	2.04
Day 7	Control	45	10.24	2.74
Day 7	PO knockdown	45	13.37	1.92

3.6. Host Survival Following *S. aureus* Infection

There were differences between the survival rates of the control and immune-knockdown groups within the 30-day monitoring period. In the AMP-knockdown experiment, mortality increased from 112/300 (37.3%) in control beetles to 139/300 (46.3%) following knockdown, representing an increase of 9.0 percentage points. In the PO-knockdown experiment, mortality increased from 114/300 (38.0%) in controls to 158/300 (52.7%) following knockdown, representing an increase of 14.7 percentage points. Overall differences indicate immune suppression is associated with higher mortality, but in varying degrees and directions for different strains of the bacteria. The time course of host survival after exposure to AMP and PO knockdown is presented in Figure 3. The pooled infected groups had lower survival probabilities than their pooled controls, for both knockdown groups. These curves are descriptive and statistical significance demands appropriately specified survival comparisons.

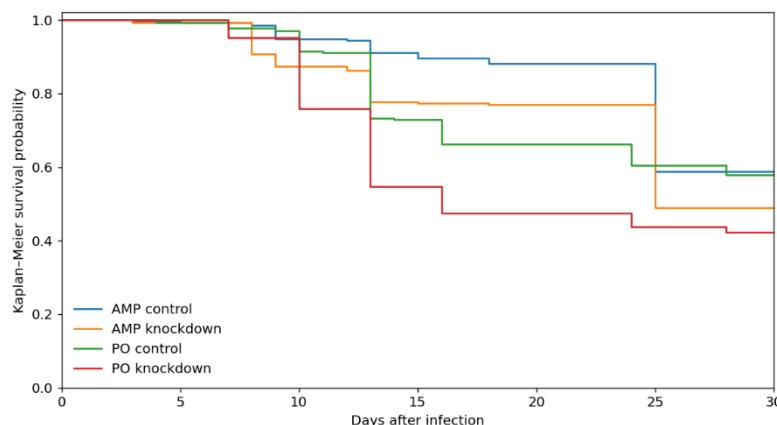


Figure 3. Kaplan–Meier Survival Curves Following *S. aureus* Infection

3.7. Influence of Antimicrobial Peptide Resistance on Infection Outcomes

AMP^r and infection outcome were dependent on the type of host immune-knockdown treatment. In the AMP survival experiment, resistant strains had 48.0% mortality in control hosts and 39.3% after AMP knockdown. Sensitive strains on the other hand, increased from 26.7% to 65.6%. The mortality rates of the resistant strains ranged between 48.0% and 51.3%, while those of the sensitive strains ranged between 40.0% and 74.4% in the PO experiment. These patterns indicate that there are heterogeneous interactions between the resistance status of the bacteria and the immune defense of the hosts. Table 4 shows differences in mortality for the different groups of bacteria that are sensitive and resistant to AMP. Strains represent different evolutionary lines and pooled comparisons are for descriptive purposes only and should not be construed as resistance effects of lines.

Table 4. Host Mortality by Bacterial Resistance Category and Immune Treatment

Experiment	Strain category	Control deaths / n	Knockdown deaths / n	Control mortality (%)	Knockdown mortality (%)
AMP	Sensitive	24/90	59/90	26.7	65.6
AMP	Resistant	72/150	59/150	48.0	39.3
PO	Sensitive	36/90	67/90	40.0	74.4
PO	Resistant	72/150	77/150	48.0	51.3

3.8. Integrated Assessment of Bacterial Resistance and Host Immune Responses

In the integrated analysis, relationships were detected between the type of resistance of the bacteria, immune suppression, bacterial persistence and host survival. A higher pooled median bacterial burden on Day 7 was correlated with the knockdown of the AMP gene, and both AMP and PO knockdown were associated with increased total mortality. However, there was a wide range of individual strain responses, suggesting that resistance status to the bacteria was not always a reliable indicator of the severity of infection. The integration of Day 7 bacterial loads and 30-day mortality rates yielded a descriptive comparison of bacterial persistence and infection outcomes by strain and immune treatments. The experimental strains and treatments were analyzed for variation in bacterial burden and host mortality, shown in Figure 4. The heterogeneous distribution suggests that increased counts of bacteria did not consistently lead to increased mortality. The figure shows group-level associations, rather than individual-level associations, as bacterial-load and survival observations were made at different experimental beetles.

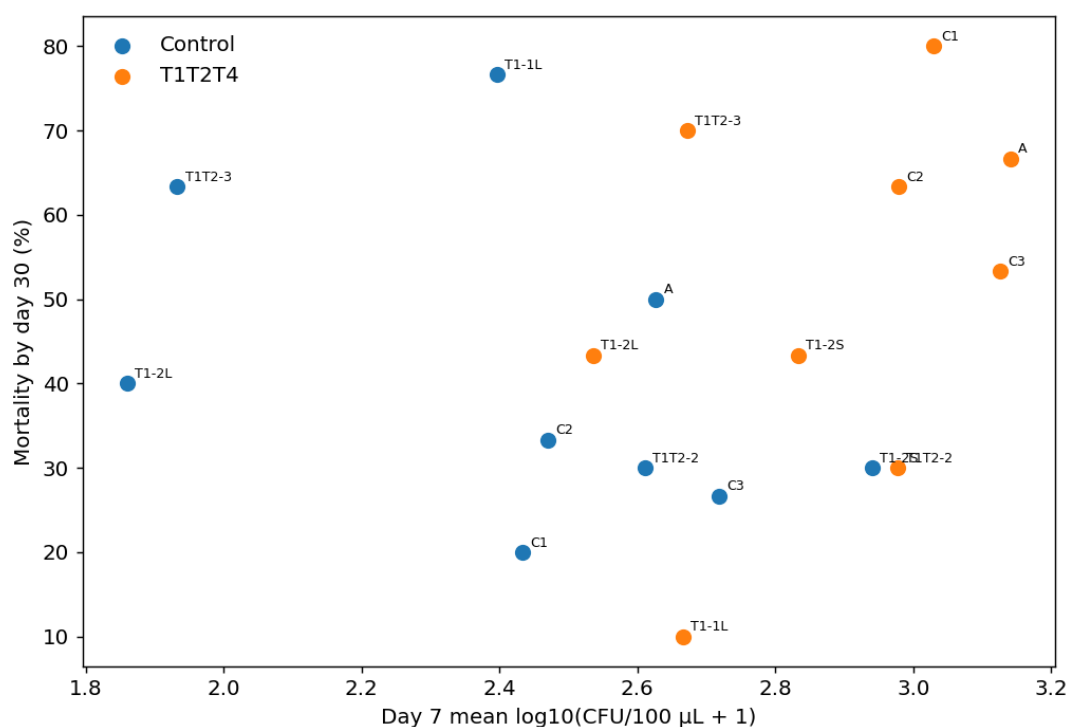


Figure 4. Relationship Between Bacterial Burden and Host Mortality Across *S. aureus* Strains

4. Discussion

This study investigated antimicrobial peptide (AMP) resistance and host immune response during infection with *Staphylococcus aureus* using an insect host model. The results showed a significant difference in bacterial burden, expression of immune genes, and host survival among experimental treatments. Bacterial persistence on Day 7 was related to AMP knockdown, whereas AMP and prophenoloxidase (PO) knockdown enhanced overall mortality. But there were significant differences in the outcome of infection with different bacterial strains, which demonstrates that bacterial resistance and host defense interact in a complex manner. These findings support the importance of evolutionary adaptation in determining antimicrobial effectiveness and host–pathogen interactions (Lazzaro et al., 2020; Mookherjee et al., 2020).

The AMP-resistant *S. aureus* variants had heterogeneous infection outcomes relative to AMP-sensitive strains, indicating that classification of resistance is not always indicative of bacterial persistence or pathogenicity. The pooled bacterial loads decreased between Days 1 and 7, but increased on Day 7 after AMP knockdown, suggesting a decrease in bacterial clearance under suppressed immune circumstances. These findings align with evolutionary views highlighting the importance of resistance development in modifying bacterial fitness and antimicrobial defense interactions with host antimicrobial defenses (Yu et al., 2018). Moreover, antimicrobial peptide adaptation by bacteria can be associated with compensatory and resistance-related physiological alterations affecting the survival of pathogens (Baindara et al., 2020). This complexity makes it essential to consider resistance in the context of biological infections, as opposed to using traditional susceptibility measures only.

The expression of the tenecin and PO genes was reduced after knockdown, hence highlighting their role in insect innate immune responses. Reduced normalized expression of T1, T2, and T4 was coupled with higher bacterial persistence at the end sampling point, which is consistent with the protective nature of antimicrobial peptide activity. An earlier study found immune-related gene-expression responses as key elements of *Tenebrio molitor* responses to microbial hostilities (Petronio Petronio et al., 2022). The immune defense mechanism helps in regulating antimicrobial defense, and autophagy offers additional defense against invading microorganisms (Jang et al., 2022; Jo et al., 2021). However, the use of transcriptional measurements does not suffice to determine equivalent changes in peptide abundance or antimicrobial activity.

The bias in immune suppression affected bacterial persistence and survival, but not equally among bacterial strains. Both AMP and PO knockdown were associated with increased overall host mortality. Mortality increased by 9.0 percentage points following AMP knockdown and 14.7 percentage points following PO knockdown, indicating a greater descriptive mortality increase in the PO-knockdown experiment. The increase in bacterial load after the AMP knockdown is an indication of poor infection control, and the strain-level comparisons showed that the bacterial load and mortality were not always proportional. The difference underlines complications of infection outcomes that rely on bacterial virulence and various processes of host defense. The studies of *S. aureus* infections also highlight the significance of integrated immune responses in the eradication of pathogens and defense of tissues (Park & Liu, 2021; Pidwill et al., 2021).

These results are in line with the studies that have shown that the host-defense peptides play a role in antimicrobial defense but are remarkably functionally diverse. The study by Cecotto et al. found that host-defense peptides have antibacterial and anti-inflammatory effects against *S. aureus*, which justifies their role in infection control (Cecotto et al., 2022). Newer developments in engineered antimicrobial peptides have also exhibited the potential of peptide-based approaches to resist bacterial infections (Lakshmaiah Narayana et al., 2021). Likewise, Zhang and Yang noted the potential of antimicrobial peptides in the treatment process and the issues related to stability, efficacy, and resistance (Zhang & Yang, 2022). The current results build on those views by demonstrating the relationship between infection outcome and the immune suppression/bacterial resistance status, which is mediated by immune suppression in an insect host.

The insect infection model could not directly generalize to human immune responses, and resistance classifications could not be matched with minimum inhibitory concentration measurements. And the separate experimental data sets could not be integrated at the individual level with bacterial load, gene expression, and survival. Also, lack of observations, missing qPCR group identifiers and replication of evolutionary lines limited interpretation. Future studies ought to include increased biological replication, confirmed experimental identifiers, antimicrobial susceptibility, and peptide-level functional measurements. Bacterial burden and immune activity longitudinal studies coupled with survival further explain resistance-related pathogenic processes and enhance insights into host–pathogen interactions.

5. Conclusion

The present study assessed antimicrobial peptide resistance and host immune responses in *Staphylococcus aureus* infection based on experimental data generated from the *T. molitor* insect host model. The results indicated that there was a large difference in persistence, immune-gene expression, and host survival between antimicrobial peptide-resistant and sensitive strains of bacteria. The knockdown of antimicrobial peptides correlated with higher bacterial counts at the late infection time point, suggesting that innate immunity is essential to limit the growth of bacteria. The down-regulation of tenecin 1, tenecin 2, tenecin 4, and prophenoloxidase was corroborated by the transcriptional changes that were associated with immune suppression. In addition, the mortality of the overall hosts was higher after the knockdown of antimicrobial peptides and prophenoloxidase, but varied significantly between bacterial strains. Taken together, these observations indicate that antimicrobial peptide resistance is not a sole determinant of infection severity, but rather acts in conjunction with host immune factors and bacterial traits. The varied correlation between bacterial load and death rates once again highlights the intricacies of host–pathogen dynamics. However, the use of the insect experimental design, limited evolutionary-line replication, and lack of direct antimicrobial susceptibility measurement limit the conclusions regarding clinical interpretation. Future studies need to include more experimental replication, molecular resistance mechanisms and integrated evaluation of immune activity and bacterial persistence. Overall, this study helps to understand the relationship

between antimicrobial peptide resistance and host innate immunity and serves as a baseline for future antimicrobial resistance and host defense studies.

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