Official Journal of Erciyes University Faculty of Medicine

DOI: 10.14744/cpr.2025.96095
J Clin Pract Res 2025;47(2):223–226

In vitro Antimicrobial Activities of Temporin A and Apidaecin B Peptides Against Clinical Strains Isolated from Blood Culture

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Cite this article as:
Yayla İ, Aydoğan O, Kılıç A, Sirekbasan L, İstanbullu Tosun A, Kocazeybek B, Dinç HÖ. *In vitro* Antimicrobial Activities of Temporin A and Apidaecin B Peptides Against Clinical Strains Isolated from Blood Culture. JClinPractRes2025;47(2):223–226.

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Submitted: 09.10.2024
Revised: 14.10.2024
Accepted: 10.03.2025
Available Online: 24.03.2025

Erciyes University Faculty of Medicine Publications - Available online at www.jcpres.com

ABSTRACT

The increase in antimicrobial resistance poses a significant challenge in medicine, necessitating the exploration of novel antimicrobial agents. Temporin A and Apidaecin B, two naturally occurring antimicrobial peptides (AMPs), have garnered attention for their potent bactericidal properties. This study aimed to investigate the antimicrobial activities of Temporin A and Apidaecin B against clinical strains isolated from blood cultures and evaluate their potential as alternative therapeutic agents. A total of 12 *Escherichia coli*, 13 *Klebsiella pneumoniae*, 13 *Staphylococcus aureus*, and 12 *Staphylococcus epidermidis* strains were used for antimicrobial susceptibility tests. Antimicrobial activities were evaluated using the broth microdilution method. MIC values were detected as <3.9 for Temporin A against *S. aureus* and *S. epidermidis* and 7.81 for Apidaecin B against *E. coli*. The results underscore the potent antimicrobial properties of Temporin A against *S. aureus* and *S. epidermidis*, and Apidaecin B against *E. coli*. Further research is needed to optimize AMPs' stability, delivery mechanisms, and efficacy *in vivo*.

Keywords: Apidaecin B, *E. coli*, *S. aureus*, *S. epidermidis*, Temporin A.

INTRODUCTION

Bloodstream infections (BSIs) are particularly concerning when caused by multidrug-resistant (MDR) pathogens, which pose significant challenges in clinical treatment. The rapid emergence of antimicrobial resistance complicates therapeutic options, emphasizing the urgent need for alternative antimicrobial agents. One promising avenue of research involves antimicrobial peptides (AMPs), which are integral to the innate immune response and can neutralize a wide range of bacterial species through diverse mechanisms, including disruption of bacterial membranes and interference with essential cellular processes.[1]

Among the most promising AMPs under investigation are Temporin A and Apidaecin B. Temporin A, derived from amphibian skin, is noted for its rapid action and heat stability, making it effective against both Gram-positive and Gram-negative bacteria. It has shown potent activity against antibiotic-resistant strains, highlighting its potential as a new therapeutic agent.[2]

Apidaecin B, isolated from honeybee hemolymph, specifically targets Gram-negative bacteria, exhibiting strong antimicrobial activity against important pathogens such as *Escherichia coli*. This peptide works by binding to bacterial cell walls and disrupting their integrity, thereby inhibiting bacterial growth.[3,4]

Given the wide variability in the geographical distribution and resistance patterns of BSI-causing organisms, it is crucial to explore AMPs that target multiple bacterial species. *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* are among the most frequent pathogens implicated in BSIs, each presenting distinct challenges to effective treatment.[5] MRSA has become a major healthcare burden due to its resistance to conventional antibiotic therapies. Additionally, the biofilm-forming capacity of *Staphylococcus epidermidis* complicates the clinical management of catheter-associated and other device-related infections, necessitating innovative treatment approaches.[6]

This study aims to investigate the *in vitro* antimicrobial efficacy of these two AMPs against clinical isolates of *S. aureus*, *E. coli*, *K. pneumoniae*, and *S. epidermidis* isolated from blood culture samples, offering insights into their potential clinical applications as next-generation antimicrobial agents.

MATERIALS AND METHODS

Ethics

The study was approved by the İstanbul Medipol University Ethics Committee (Date: 13.10.2022, Decision Number: 844). The study adhered to the principles of the Declaration of Helsinki.

Bacterial Strains, Culture Conditions, and Reagents

Escherichia coli ATCC®35218, *Klebsiella pneumoniae* ATCC®33495, *Staphylococcus aureus* ATCC®29213, and *Staphylococcus epidermidis* ATCC®49461 were obtained from Üsküdar University, Department of Medical Microbiology. All strains were grown in Nutrient Agar medium and then cultured in Mueller-Hinton agar medium at 37°C.

A total of 12 *E. coli*, 13 *K. pneumoniae*, 13 *S. aureus*, and 12 *S. epidermidis* strains were used for antimicrobial susceptibility tests, which were isolated from blood cultures. All clinical samples were obtained from the Istanbul Medipol University, Medipol Mega University Hospital, Medical Microbiology Laboratory. and identified using MALDI-TOF MS (Bruker). Ceftriaxone and vancomycin were used as control antimicrobial agents.

Antimicrobial Peptides (AMPs)

Temporin A and Apidaecin B were used as tested antimicrobial peptides (Temporin A, amide, AnaSpec Inc., USA; Apidaecin B, AnaSpec Inc., USA). For Temporin A and Apidaecin B, 1 mg of AMP was dissolved in 1 mL of 0.22 µm membrane-filtered distilled water.

Kirby-Bauer Disc Diffusion Method

To conduct the test, bacterial cultures were extracted from 0.5 McFarland tubes prepared for each tested sample. All bacteria were streaked on Mueller-Hinton Agar (BD BBL; Becton, Dickinson and Company, NJ, USA). Empty discs in sterile petri dishes received 20 µg of AMPs. After 24 hours, the zone diameters were measured.

Determination of Minimum Inhibitory Concentrations (MICs)

The antimicrobial activities of the peptides were evaluated using the broth microdilution method. MIC determinations for the peptides were performed in Mueller-Hinton broth (Becton Dickinson, NJ, USA) by the broth microdilution method following EUCAST guidelines. A 96-well microtiter plate was used for antimicrobial susceptibility testing (AST). Peptide concentrations were inoculated in the 0.5 McFarland (diluted 1:10). After 24 hours of incubation at 37°C, the MICs of wells were recorded. These tests were conducted twice at different times.

A 0.5 McFarland saline broth was prepared with the bacteria, and the test was performed in 96-well U-bottom microplates. Each well received 100 µL of bacterial broth solution and 100 µL of AMP solution. After making two-fold dilutions, the concentrations were prepared (500 µg/mL, 250 µg/mL, 125 µg/mL...). A total of 100 µL of vancomycin was used in the control well. The inoculum was diluted in McFarland 0.5 standard turbidity (1:10). Within 15 minutes, 20 µL was added to all wells except the sterile well, and then the plates were incubated.

RESULTS

Kirby-Bauer Disc Diffusion Test

Following 24 hours of incubation, the disc diffusion test with *S. aureus* (ATCC 29213) and Temporin A resulted in a zone of inhibition measuring 14 mm. In contrast, no zone of inhibition was observed for *E. coli* (ATCC 35218) treated with Temporin A after the same period (Fig. 1). For *K. pneumoniae* (ATCC 33495), ciprofloxacin produced a 17 mm zone, while Apidaecin B exhibited a modest zone of 8 mm. For *S. aureus* (ATCC 29213), Temporin A produced a zone of 22 mm, whereas Apidaecin B had no detectable antimicrobial activity. In *E. coli* (ATCC 35218), Temporin A displayed

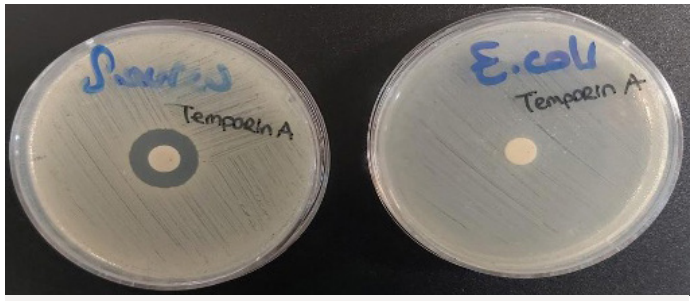

Figure 1. Kirby-Bauer disc diffusion method results for *S. aureus* (ATCC 29213) and *E. coli* (ATCC 35218).

Table 1. MIC values for AMPs

	Apidaecin B	Temporin A
S. aureus	–	<3.9 µg/mL
S. epidermidis	–	<3.9 µg/mL
E. coli	7.81 µg/mL	–
K. pneumoniae	62.5 µg/mL	125 µg/mL

significant antibacterial activity, producing a 35 mm zone, while Apidaecin B formed a 16 mm zone.

Minimum Inhibitory Concentration Test

In this study, the antimicrobial activities of the peptides Temporin A and Apidaecin B were evaluated against a total of 50 clinical strains isolated from blood cultures. MICs of both peptides were determined using standard broth microdilution methods. All MIC values for AMPs are shown in Table 1.

Temporin A Activity

Temporin A exhibited potent antimicrobial activity against all tested staphylococcal strains. The MIC values for *S. aureus* and *S. epidermidis* were <3.9 µg/mL, with MRSA strains showing slightly higher MIC values compared to methicillin-sensitive strains. *K. pneumoniae* strains were inhibited by Temporin A at high MIC values (64–125 µg/mL). Temporin A had no antimicrobial activity against *E. coli in vitro*. Overall, Temporin A demonstrated broad-spectrum activity, although some variability in susceptibility was observed among different bacterial species.

Apidaecin B Activity

Apidaecin B also showed significant antimicrobial activity against the tested Gram-negative clinical strains, though its efficacy was generally lower than that of Temporin A, especially in Gram-positive ones. The MIC values for Gram-negative strains were between 7.81 µg/mL and 62.5 µg/mL, with *K. pneumoniae* strains showing higher resistance compared to *E. coli* strains. Apidaecin B had no antimicrobial activity against all *S. aureus* and *S. epidermidis* strains *in vitro*.

DISCUSSION

The results of this study reveal that both Temporin A and Apidaecin B hold significant promise as future antimicrobial agents, with Temporin A showing particular efficacy against Gram-positive strains such as *Staphylococcus aureus* and *Staphylococcus epidermidis*. The ability of Temporin A to act against biofilm-producing organisms like *S. epidermidis* could have significant implications for managing device-related infections, which are notoriously difficult to treat with standard antibiotics.[7,8] Furthermore, Apidaecin B, while less effective against Gram-positive bacteria, demonstrated robust activity against Gram-negative strains. Importantly, our study aligns with previous research, which has similarly demonstrated the selective efficacy of Temporin A against Gram-positive bacteria and Apidaecin B against Gram-negative strains.[8–11]

Similar to our study, *in vitro* antimicrobial activity of Apidaecin B on Gram-negative bacteria and Temporin A on Gram-positive bacteria has been reported in the literatüre.[9] This consistency supports the validity of our findings and confirms the potential of these peptides for future therapeutic applications. Li et al.[10] demonstrated that proline-rich antimicrobial peptides, such as Apidaecin, exhibit *in vitro* antimicrobial and antibiofilm effects against various Gram-negative bacteria. Moreover, it has been reported that not only Temporin A but also other Temporin peptides show strong antimicrobial activity against Gram-positive bacteria.[11]

Swithenbank et al.,[12] in their meta-analysis study evaluating the potential clinical applications of AMP-coated implants, demonstrated that coating foreign bodies such as implants with AMPs exhibits effective antimicrobial activity. Their study found that the most commonly targeted bacterium was *S. aureus*, and the most frequently used AMP was HHC36. We also believe that planning *in vivo* studies involving AMP-coated implants, including Temporin and Apidaecin, is crucial for preventing foreign body infections.[12]

The discovery and synthesis of novel AMPs should be supported by integrating both clinical research and advanced computational approaches, including artificial intelligence and machine learning. For instance, Santos-Júnior et al.[13] employed machine learning to predict the activity of 100 AMPs, which were subsequently synthesized and tested against clinically relevant MDR pathogens and human intestinal commensals in both *in vitro* and *in vivo* models, validating their computational predictions.

Our findings align with previous studies that investigated the antimicrobial efficacy of AMPs against various bacterial species, demonstrating their potential as alternative therapeutic agents.[14] The antimicrobial activity of hybrid AMPs synthesized

by merging honeybee-derived Apidaecins with frog-derived Temporins has been investigated in a study conducted in Türkiye, demonstrating promising antibacterial effects against both Gram-positive and Gram-negative bacteria.[15]

CONCLUSION

While Temporin A and Apidaecin B exhibited notable antibacterial activity, particularly against *S. aureus*, *S. epidermidis*, and *E. coli*, their efficacy remains comparable to, but not superior to, commercially available antibiotics. These findings suggest that although these peptides hold promise as alternative antimicrobial agents, further research is required to enhance their potency, optimize delivery systems, and evaluate their *in vivo* efficacy. Additionally, comparative studies with other AMPs, particularly those reported in the literature from different regions, could provide valuable insights into their therapeutic potential. Future research should focus on structural modifications to improve stability, bioavailability, and selectivity while also exploring synergistic interactions with conventional antibiotics.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Author Contributions: Concept – BK, AİT, HÖD; Design – BK, AİT, HÖD; Supervision – BK, AİT, HÖD; Resource – AİT, LS, OA, AK, İY, HÖD; Materials – AİT, LS, OA, AK, İY, HÖD; Data Collection and/or Processing – LS, AK, OA; Analysis and/or Interpretation – BK, AİT, HÖD, İY; Literature Search – İY, LS, OA, AK; Writing – İY; Critical Reviews – İY, OA, AK, LS, AİT, BK, HÖD.

Conflict of Interest: The authors have no conflict of interest to declare.

Use of AI for Writing Assistance: Artificial Intelligence was not used for writing assistance.

Financial Disclosure: The authors declare that this study has been supported by Bezmialem Vakıf University, Scientific Research Projects Unit (Project ID: 20221206).

Peer-review: Externally peer-reviewed.

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