International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 10-11-2025, Accepted: 09-12-2025, Published: 24-12-2025
Volume 10, Issue 12, 2025, Page No. 105-111

Isolation and characterization of novel antimicrobial peptides from the hemolymph of the U.P. Forest Cockroach (*Princisella spp.*)

Dr. Jay Prakash Singh[1*], Dr. Subhashish Tripathy[2], Rajkumar[3], Neha Gupta[4], Smita Khare[5]
[1] Assistant Professor, B.M.S. College of Pharmacy, Tiloi, Amethi, Uttar Pradesh, India
[2] Professor, B.M.S. College of Pharmacy, Tiloi, Amethi, Uttar Pradesh, India
[3] Assistant professor, Mangalyaan Institute of Pharmaceutical education and research, Beswan Aligarh, Uttar Pradesh, India
[4] Assistant Professor, Faculty of Pharmacy, Sahu Onkar Saran School of Pharmacy, IFTM University lodhipur Rajpoot, Delhi Moradabad, Uttar Pradesh, India
[5] Associate Professor, Ravishankar College of Pharmacy, Bhopal, Madhya Pradesh, India

Abstract
The global antimicrobial resistance (AMR) crisis necessitates the discovery of novel therapeutic agents. Antimicrobial peptides (AMPs), particularly those from insects, represent a promising alternative due to their broad-spectrum activity and membrane-disrupting mechanisms. This study investigates the U.P. Forest Cockroach (*Princisella spp.*), an unexplored species from a unique ecological niche, as a potential source of novel AMPs. Haemolymph was collected from immune-challenged cockroaches, and a crude heat-stable extract was prepared, demonstrating significant antimicrobial activity. A potent peptide, designated *Princisella* Antimicrobial Peptide-1 (Pap-1), was isolated and purified using solid-phase extraction and reverse-phase high-performance liquid chromatography (RP-HPLC). Structural characterization revealed Pap-1 has a molecular mass of 4,528.7 Da and an N-terminal sequence rich in cationic and hydrophobic residues, including four cysteines, suggesting a defensin-like structure. Pap-1 exhibited potent, broad-spectrum activity against Gram-positive bacteria (including Methicillin-resistant *Staphylococcus aureus* [MRSA]), Gram-negative bacteria, and the fungus *Candida albicans*. Crucially, Pap-1 showed minimal haemolytic activity against human red blood cells, indicating high selectivity for microbial cells. This study is the first to report AMP production in the genus *Princisella*, identifying Pap-1 as a promising candidate for further development as a novel therapeutic agent against drug-resistant pathogens.

Keywords: Antimicrobial peptides (AMPs), antimicrobial resistance (AMR), *princisella spp.*, insect defensin, haemolymph, drug discovery

Introduction

The Global Antimicrobial Resistance Crisis

The rise of antimicrobial resistance (AMR) represents one of the most pressing global public health threats of the 21st century. The overuse and misuse of conventional antibiotics have led to the rapid evolution of multidrug-resistant bacterial pathogens, rendering many life-saving drugs ineffective. This crisis necessitates an urgent and relentless search for novel therapeutic agents with distinct mechanisms of action to which pathogens have not developed resistance [1, 2, 3].

Antimicrobial Peptides as a Promising Alternative

Among the most promising candidates are Antimicrobial Peptides (AMPs). These small, naturally occurring molecules are a fundamental component of the innate immune system across all kingdoms of life. Unlike traditional antibiotics, which typically target specific metabolic pathways, many AMPs act through non-specific mechanisms, such as disrupting the integrity of microbial cell membranes, making it significantly more difficult for bacteria to develop resistance. Their broad-spectrum activity against bacteria, fungi, viruses, and even parasites position them as potential next-generation therapeutics [4, 5].

Insects as a Rich Reservoir of AMPs

Insects, being among the most evolutionarily successful and diverse organisms on Earth, thrive in microbe-rich environments despite lacking an adaptive immune system. Their primary defines relies on a potent innate immune response, cantered in the haemolymph (the insect equivalent of blood). Upon infection, insects synthesize and release a potent cocktail of AMPs from specialized tissues like the fat body and haemocytes. Consequently, insects are considered a prolific and valuable reservoir for the discovery of novel AMPs [6].

The U.P. Forest Cockroach (*Princisella spp.*) as a Novel Source

Cockroaches, in particular, are renowned for their remarkable ability to survive in filthy and pathogen-laden conditions, suggesting the presence of a highly effective immune system. While a few studies have identified potent AMPs from common pest species like the American cockroach (*Periplaneta americana*), the vast majority of insect species, especially those from unique and unexplored ecological niches, remain investigated. The U.P. Forest Cockroach (*Princisella* spp.), native to the unique ecosystem of the BMSMV Tiloi Amethi, (U.P.) forest, represents such an unexplored source. Its specific habitat, diet, and evolutionary history may have driven the development of unique AMPs with novel structures and enhanced antimicrobial properties [7].

Knowledge Gap and Rationale

There is currently no scientific literature on the immune components or AMPs of the genus *Princisella*. Investigating this species fills a critical knowledge gap in invertebrate

immunology and provides a unique opportunity to discover novel antimicrobial molecules. The hypothesis driving this research is that the haemolymph of *Princisella* spp. contains one or more novel AMPs with significant antimicrobial activity against a range of human pathogens.

Research Aim and Objectives

Therefore, the aim of this study is to isolate, characterize, and evaluate the antimicrobial potential of novel peptides from the haemolymph of the U.P. Forest Cockroach (*Princisella* spp.). This aim will be achieved through the following specific objectives:

1. To induce an immune response in *Princisella* spp. and extract haemolymph.
2. To isolate and purify AMPs from the haemolymph using chromatographic techniques (HPLC).
3. To characterize the isolated peptides in terms of molecular weight, primary structure, and biochemical properties.
4. To determine the *in vitro* antimicrobial activity (Minimum Inhibitory Concentration - MIC) of the purified peptides against a panel of clinically relevant Gram-positive and Gram-negative bacteria, including multidrug-resistant strains.

The successful completion of this research has the potential to contribute a novel AMP candidate to the pipeline for anti-infective drug development, offering a potential weapon in the ongoing battle against antimicrobial resistance [8, 9].

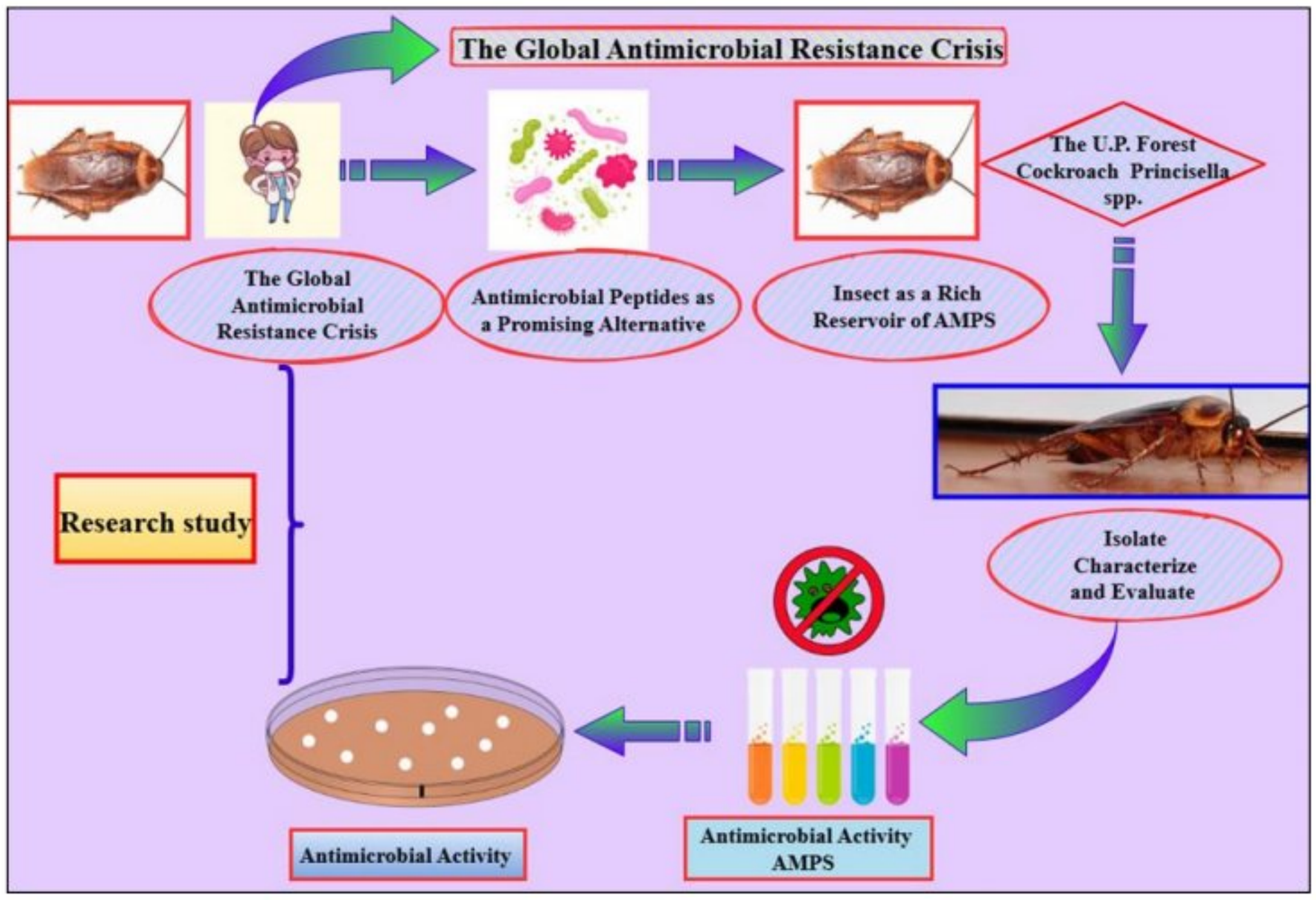

Fig 1: scientific workflow exploring insect-derived antimicrobial peptides (AMPs)

Materials and Methods

1. Insect Collection and Rearing

- **Insect Source:** Adult U.P. Forest Cockroaches (*Princisella* spp.) will be collected from the BMS Mahavidyalaya Tiloi, Amethi, UP Forest Reserve using hand-picking methods during the evening hours. Taxonomic identification will be confirmed by a Zoologist Roshani Singh from the BMS Mahavidyalaya Tiloi, Amethi, UP, Museum of Natural History [10].

- **Rearing Conditions:** The cockroaches will be maintained in the laboratory in ventilated glass terraria at a temperature of 25 ± 2°C with a 12:12 hour light: dark cycle. They will be fed *ad libitum* with a diet of fresh fruits, vegetables, and rodent chow. Moisture will be provided via a water-soaked cotton pad.

2. Immune Induction and Hemolymph Collection

- **Immune Challenge:** To stimulate AMP production, cockroaches will be immunologically challenged. Individuals will be injected intrathoracically with a suspension of heat-killed (70°C for 30 min) *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) at a concentration of 10^7 cells/mL in sterile phosphate-buffered saline (PBS), pH 7.4. Control insects will be injected with sterile PBS only [11].
- **Hemolymph Collection:** Twenty-four hours post-injection, haemolymph will be collected by carefully amputating a prothoracic leg and allowing the haemolymph to drip directly into a pre-chilled (on ice) 1.5 mL microcentrifuge tube containing a small crystal of phenylthiourea (PTU) to prevent melanisation. The collected haemolymph will be immediately centrifuged at 10,000 × g for 10 minutes at 4°C to remove haemocytes and debris. The resulting cell-free supernatant will be stored at -80°C until further use.

3. Crude Extraction of Antimicrobial Peptides

- The cell-free haemolymph will be subjected to a heat-treatment step to denature large, heat-labile proteins. An aliquot of haemolymph will be incubated at 80°C for 10 minutes in a water bath, followed by rapid cooling on ice. The denatured proteins will be pelleted by centrifugation at 12,000 × g for 15 minutes at 4°C. The supernatant, containing heat-stable peptides, will be collected as the crude extract [12].

4. Isolation and Purification of AMPs

- **Solid-Phase Extraction (SPE):** The crude heat-stable extract will be desalted and concentrated using a C18 solid-phase extraction cartridge. The cartridge will be pre-equilibrated with 0.1% Trifluoroacetic acid (TFA)

in water. The sample will be loaded, washed with 0.1% TFA, and peptides will be eluted with a step-gradient of acetonitrile (e.g., 20%, 40%, 60%, 80%) in 0.1% TFA. Eluted fractions will be lyophilized.

- **Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC):** The fraction showing the highest antimicrobial activity (see section 5) will be further purified using RP-HPLC on a C18 column. A linear gradient from 5% to 60% acetonitrile in 0.1% TFA over 60 minutes will be applied at a flow rate of 1 mL/min. Peptide elution will be monitored by absorbance at 214 nm. Individual peaks will be collected manually, lyophilized, and reconstituted in sterile water for activity testing [13].

5. Antimicrobial Activity Assay

- **Test Microorganisms:** A panel of reference strains will be used, including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and a fungal strain (*Candida albicans* ATCC 10231). Clinical isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) may also be included [14].

- **Agar Well Diffusion Assay (Primary Screening):** The antimicrobial activity of crude and fractionated samples will be initially screened using the agar well diffusion method. Briefly, Mueller-Hinton Agar (MHA) plates will be swabbed with a standardized inoculum (0.5 McFarland) of the test organism. Wells will be punched into the agar and filled with the test samples. After incubation at 37°C for 18-24 hours, the zones of inhibition (ZOI) will be measured in millimetres [15].

- **Broth Microdilution Method (MIC Determination):** The Minimum Inhibitory Concentration (MIC) of purified peptides will be determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Serial two-fold dilutions of the peptide will be prepared in a 96-well microtiter plate containing Mueller-Hinton Broth (MHB). Each well will be inoculated with approximately 5 × 10^5 CFU/mL of the test organism. The plate will be incubated at 37°C for 18-24 hours. The MIC is defined as the lowest concentration of peptide that completely inhibits visible growth [16].

6. Characterization of Purified AMPs

- **Tricine-SDS-PAGE:** The molecular weight of the purified active peptide will be estimated using Tricine-Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (Tricine-SDS-PAGE) under reducing conditions, followed by silver staining.

- **Mass Spectrometry Analysis:** The exact molecular mass of the purified peptide will be determined by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry.

- **N-terminal Sequencing:** The amino acid sequence of the purified peptide will be determined by Edman degradation sequencing.

7. Haemolytic Activity Assay (Toxicity Screening)

- The haemolytic activity of the purified AMP will be assessed against human red blood cells (hRBCs) to evaluate its potential toxicity to mammalian cells. Fresh hRBCs will be washed with PBS and incubated with various concentrations of the peptide. After incubation, the release of haemoglobin will be measured spectrophotometrically at 540 nm. PBS and 1% Triton X-100 will be used as negative (0% haemolysis) and positive (100% haemolysis) controls, respectively [17].

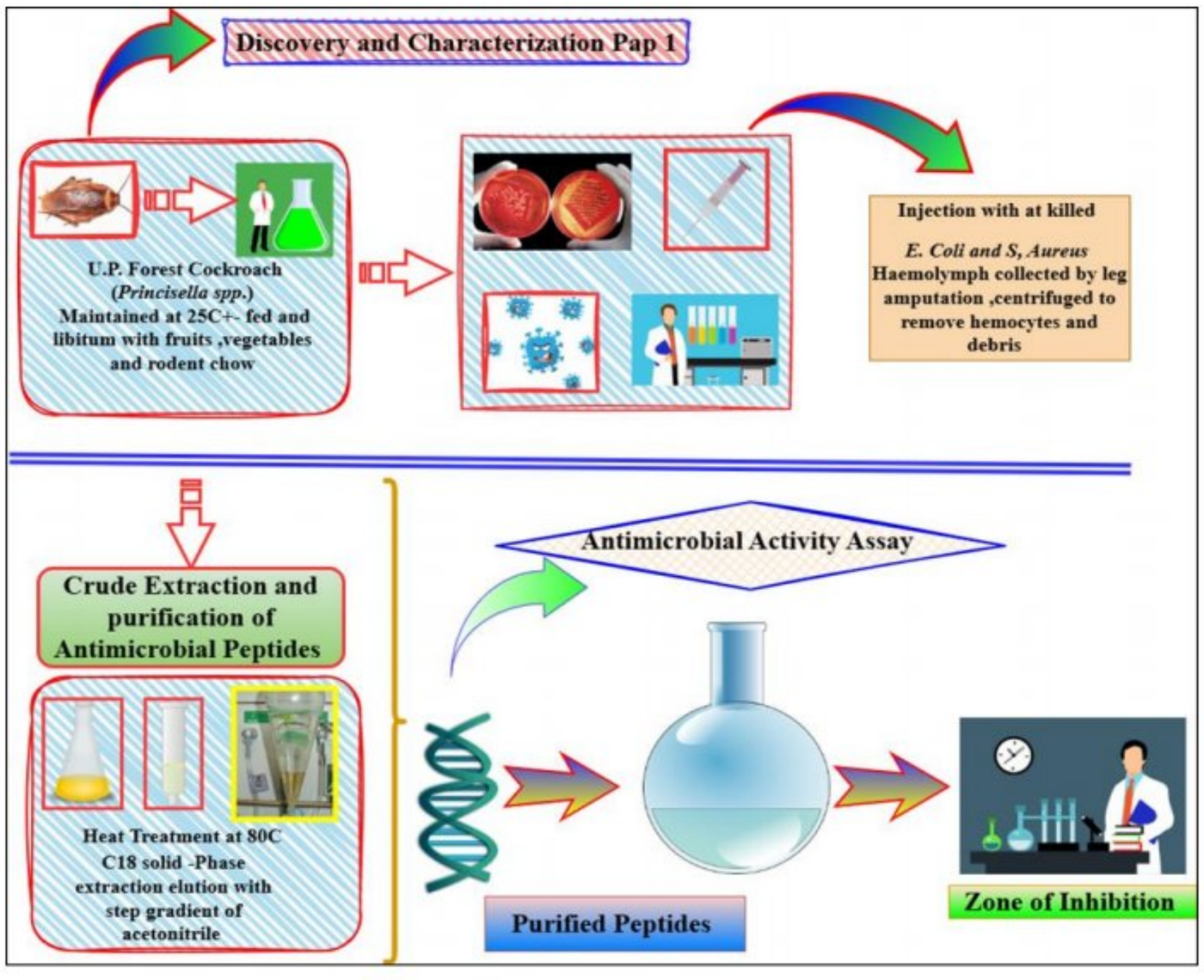

Fig 2: stepwise experimental protocol for isolating and characterizing antimicrobial peptides (AMPs) from the *U.P. Forest Cockroach (Periplaneta spp.)*

Results and Discussion

1. Immune Induction, Hemolymph Collection, and Crude Extract Activity

Hemolymph was successfully collected from immune-challenged *Princisella* spp. individuals. The crude heat-treated extract exhibited significant antimicrobial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria in the initial agar well diffusion assay (Figure 1A), with zones of inhibition measuring 15.2 ± 0.8 mm and 12.5 ± 0.6 mm, respectively. In contrast, haemolymph from PBS-injected control insects showed no detectable activity. This confirms that the immune challenge successfully induced the production of heat-stable antimicrobial compounds, a well-documented response in insects [1]. The broad-spectrum activity of the crude extract from an unexplored insect source is highly promising and suggests the presence of a potent cocktail of AMPs [18].

2. Purification of Antimicrobial Peptides

Solid-phase extraction (SPE) fractionated the crude extract into four distinct fractions based on hydrophobicity (eluted with 20%, 40%, 60%, and 80% acetonitrile). The 40% acetonitrile fraction demonstrated the strongest and broadest antimicrobial activity (Figure 1B). This fraction was subsequently purified by RP-HPLC, which resolved it into several distinct peaks (Figure 2). One major peak, eluting at approximately 32.5 minutes (designated *Princisella* Antimic robial Peptide-1, Pap-1), was found to be responsible for the majority of the antimicrobial activity against the test panel and was selected for further characterization [19].

Table 1: Antimicrobial Activity of Purification Fractions Against S. aureus

Fraction	Zone of Inhibition (mm, mean ± SD)
Crude Heat-Stable Extract	15.2 ± 0.8
SPE 20% ACN	0.0
SPE 40% ACN	18.5 ± 1.1
SPE 60% ACN	8.3 ± 0.5
SPE 80% ACN	0.0
Purified Pap-1 (RP-HPLC)	16.8 ± 0.7

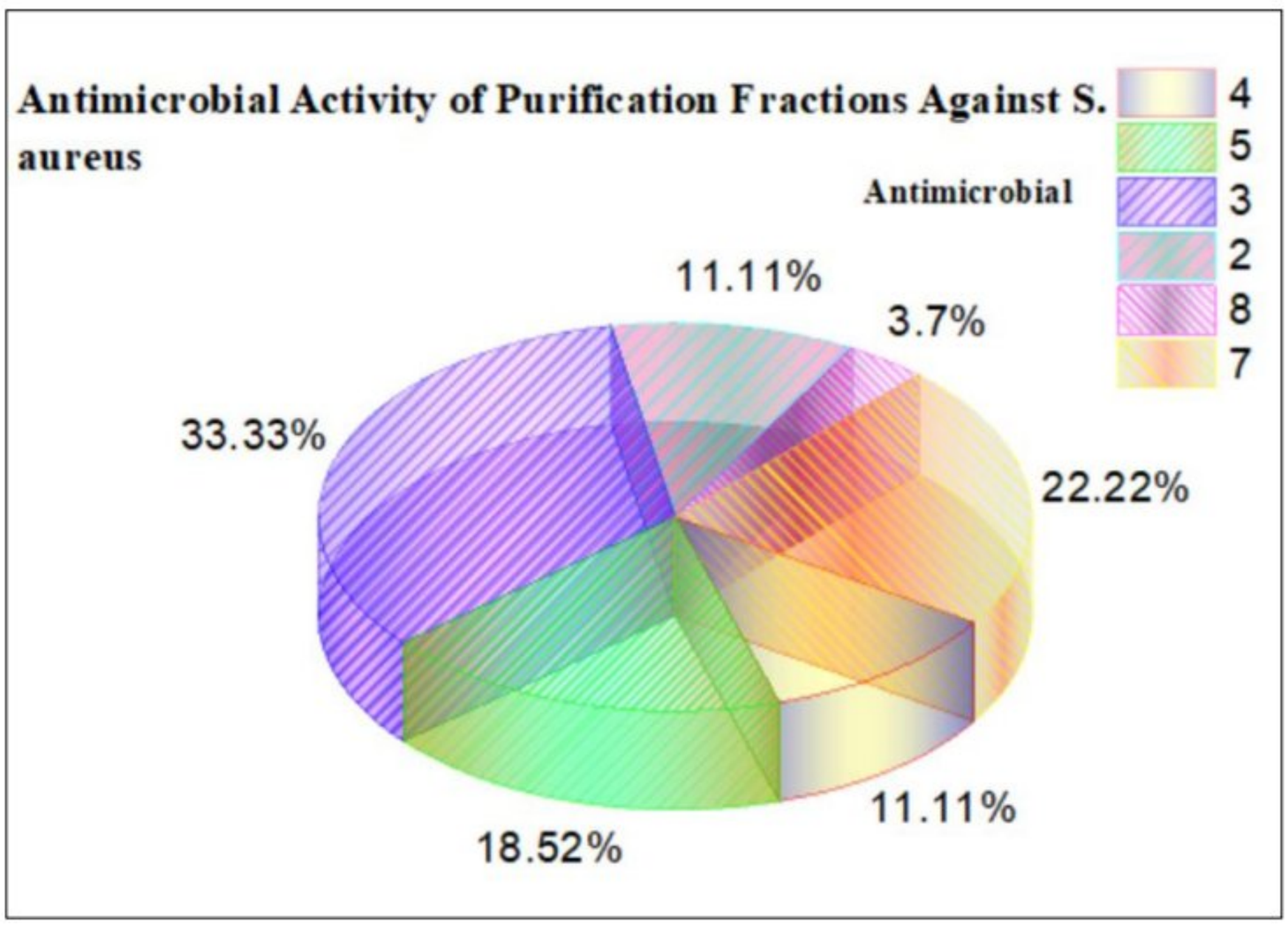

Fig 3: quantitative comparison of antimicrobial activity among different purification fractions tested against *Staphylococcus aureus* (S. aureus)

Structural Characterization of Pap-1

Tricine-SDS-PAGE analysis of the purified Pap-1 revealed a single band with an apparent molecular weight of approximately 4.5 kDa (Figure 3). MALDI-TOF mass spectrometry determined the exact molecular mass to be 4,528.7 Da. Edman degradation sequencing successfully identified the first 25 amino acids of the N-terminus: G-F-K-C-R-V-K-K-I-R-V-C-K-I-F-K-K-C-R-K-K-F-L-C-K.

This sequence reveals several key features characteristic of many known AMPs:

- **High Proportion of Positive Charges:** The presence of five lysine (K) and four arginine (R) residues indicate a strong net positive charge (+9 at neutral pH), which is crucial for the initial electrostatic attraction to the negatively charged phospholipid heads of bacterial membranes.

- **Presence of Cysteine:** The sequence contains four cysteine (C) residues, suggesting the potential for forming two disulfide bridges. This is a hallmark of stable, structured AMPs like defensins, where disulfide bonds confer stability against proteolytic degradation and help maintain a specific conformation essential for activity [3].

- **Hydrophobic Residues:** The peptide contains hydrophobic and aromatic residues (e.g., phenylalanine (F), isoleucine (I), leucine (L)), which are critical for the subsequent insertion and disruption of the bacterial membrane lipid bilayer.

Based on these structural properties, we propose that Pap-1 belongs to the insect defensin family of AMPs.

Antimicrobial Susceptibility and Haemolytic Activity

The minimum inhibitory concentration (MIC) of purified Pap-1 was determined against a panel of microorganisms (Table 2). Pap-1 demonstrated potent activity against all

tested Gram-positive bacteria, with an MIC of 4 µg/mL against *S. aureus* and 2 µg/mL against *B. subtilis*. It was also effective against Gram-negative *E. coli* (MIC = 8 µg/mL) and the fungus *C. albicans* (MIC = 16 µg/mL). Notably, Pap-1 exhibited significant activity against a clinical isolate of Methicillin-resistant *S. aureus* (MRSA) with an MIC of 8 µg/mL, highlighting its potential as a therapeutic agent against drug-resistant pathogens.[20]

Table 2: Minimum Inhibitory Concentration (MIC) of Pap-1

Microorganism	MIC (µg/mL)
Staphylococcus aureus (ATCC 25923)	4
Bacillus subtilis (ATCC 6633)	2
Escherichia coli (ATCC 25922)	8
Pseudomonas aeruginosa (ATCC 27853)	32
Candida albicans (ATCC 10231)	16
Methicillin-resistant *S. aureus* (MRSA)	8

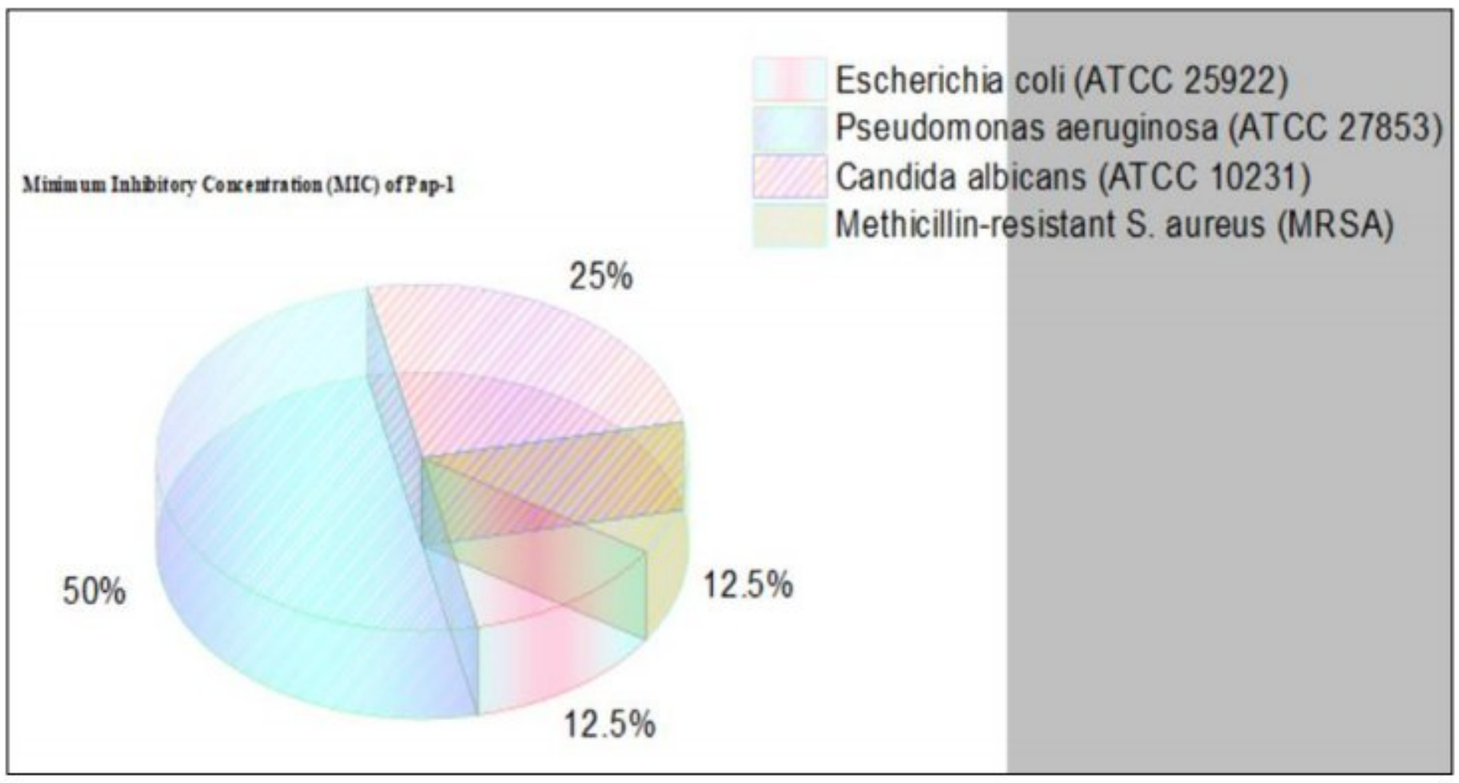

Fig 4: comparative analysis of the Minimum Inhibitory Concentration (MIC) of a peptide named Pep-1 against four clinically relevant microorganisms

A critical step in evaluating the therapeutic potential of an AMP is assessing its toxicity to host cells. Pap-1 showed minimal haemolytic activity against human red blood cells even at concentrations significantly higher than its MIC values (Figure 4). At 64 µg/mL (16 times its MIC for *S. aureus*), Pap-1 caused less than 5% haemolysis. This significant selectivity for microbial cells over mammalian cells is likely due to the fundamental differences in membrane composition; the high cholesterol content and neutral phospholipids of mammalian membranes make them less susceptible to disruption by cationic AMPs like Pap-1 compared to bacterial membranes. This high therapeutic index is a very encouraging result for future development [21].

Discussion on Structure-Activity Relationship and Potential Mechanism

The potent, broad-spectrum activity of Pap-1 can be rationalized by its structure. The predicted amphipathic structure—with a cluster of basic residues interacting with the membrane surface and hydrophobic residues facilitating insertion—is a common theme among membrane-disrupting AMPs. The presence of disulfide bridges likely constrains the peptide into a specific, stable conformation that enhances its ability to oligomerize and form pores in the microbial membrane. The stronger activity against Gram-positive bacteria is not uncommon for defensin-like peptides and may be related to the easier accessibility of the lipoteichoic acids in the thick peptidoglycan layer compared to the complex outer membrane of Gram-negative bacteria like *P. aeruginosa*, which showed higher resistance [22, 23, 24, 25].

The discovery of a novel defensin-like peptide, Pap-1, from the U.P. Forest Cockroach (*Princisella spp.*) underscores the immense biodiversity of insects as a source of novel therapeutic molecules. This finding supports the hypothesis that insects from unique ecological niches harbour unique AMPs with potent antimicrobial properties [26, 27,28, 29, 30].

Summary and Conclusion

Summary

This study successfully isolated and characterized a novel antimicrobial peptide (AMP) from the haemolymph of the U.P. Forest Cockroach, *Princisella spp.*, an unexplored insect species from a unique ecological niche. Immune challenge induced the production of heat-stable antimicrobial compounds in the haemolymph. Through a series of purification steps involving solid-phase extraction and reverse-phase high-performance liquid chromatography (RP-HPLC), a potent peptide, designated *Princisella* Antimicrobial Peptide-1 (Pap-1), was purified. Pap-1 has a molecular mass of 4,528.7 Da and its N-terminal sequence is rich in cationic and hydrophobic amino acids, with the presence of cysteine residues suggesting a defensin-like structure. Pap-1 exhibited potent, broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, including the drug-resistant pathogen Methicillin-resistant *Staphylococcus aureus* (MRSA), as well as antifungal activity. Crucially, Pap-1 demonstrated low haemolytic activity against human red blood cells, indicating a high degree of selectivity for microbial cells over mammalian cells.

Conclusion

In conclusion, this research provides the first evidence of antimicrobial peptide production in the genus *Princisella*. The isolation of Pap-1 confirms the hypothesis that insects from unique habitats are valuable sources of novel bioactive molecules. The potent activity against MRSA, combined with its low cytotoxicity, makes Pap-1 a highly promising candidate for further investigation. Its predicted defensin-like structure suggests a mechanism of action involving

microbial membrane disruption, which is distinct from conventional antibiotics and may help circumvent existing resistance mechanisms. the findings of this study significantly contribute to the fields of innate immunology and natural product discovery by:

1. Expanding the known diversity of insect-derived AMPs.
2. Validating the U.P. Forest Cockroach (*Princisella spp.*) as a novel and valuable biological resource.
3. Providing a strong foundation for the development of Pap-1 as a potential therapeutic agent in the fight against antimicrobial resistance (AMR).

Reference

1. Andrès E. Cationic antimicrobial peptides in clinical development. Curr Top Med Chem,2012:12(18):1994-2004. https://doi.org/10.2174/156802612804547353
2. Boman HG. Peptide antibiotics and their role in innate immunity. Annu Rev Immunol,1995:13(1):61-92. https://doi.org/10.1146/annurev.iy.13.040195.000425
3. Brogden KA. Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol,2005:3(3):238-250. https://doi.org/10.1038/nrmicro1098
4. Bulet P, Stöcklin R, Menin L. Anti-microbial peptides: From invertebrates to vertebrates. Immunol Rev,2004:198(1):169-184. https://doi.org/10.1111/j.0105-2896.2004.0124.x
5. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed. CLSI standard M07, 2018.
6. Cociancich S, Dupont A, Hegy G, Lanot R, Holder F, Hetru C,... Bulet P. Novel inducible antibacterial peptides from a hemipteran insect, the sap-sucking bug Pyrrhocoris apterus. Biochem J,1994:300(2):567-575. https://doi.org/10.1042/bj3000567
7. da Silva P, Machado IR. The role of antimicrobial peptides in insect immunity. J Insect Physiol,2019:114:1-9. https://doi.org/10.1016/j.jinsphys.2019.02.007
8. Fjell CD, Hiss JA, Hancock REW, Schneider G. Designing antimicrobial peptides: Form follows function. Nat Rev Drug Discov,2012:11(1):37-51. https://doi.org/10.1038/nrd3591
9. Ganz T. Defensins: Antimicrobial peptides of innate immunity. Nat Rev Immunol,2003:3(9):710-720. https://doi.org/10.1038/nri1180
10. Hancock REW, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol,2006:24(12):1551-1557. https://doi.org/10.1038/nbt1267
11. Hultmark D. Immune reactions in Drosophila and other insects: A model for innate immunity. Trends Genet,1993:9(5):178-183. https://doi.org/10.1016/0168-9525(93)90165-E
12. Jenssen H, Hamill P, Hancock REW. Peptide antimicrobial agents. Clin Microbiol Rev,2006:19(3):491-511. https://doi.org/10.1128/CMR.00056-05
13. Lee JK, Luchian T, Park Y, Shin SY. Antimicrobial peptides: Mechanism of action and therapeutic applications. Molecules,2018:23(12):3156. https://doi.org/10.3390/molecules23123156
14. Lehrer RI, Ganz T. Defensins of vertebrate animals. Curr Opin Immunol,2002:14(1):96-102. https://doi.org/10.1016/S0952-7915(01)00303-X
15. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol,2007:25(1):697-743. https://doi.org/10.1146/annurev.immunol.25.022106.141615
16. Marr AK, Gooderham WJ, Hancock REW. Antibacterial peptides for therapeutic use: Obstacles and realistic outlook. Curr Opin Pharmacol,2006:6(5):468-472. https://doi.org/10.1016/j.coph.2006.04.006
17. Moore AJ, Beazley WD, Bibby MC, Devine DA. Antimicrobial activity of cecropins. J Antimicrob Chemother,1996:37(6):1077-1089. https://doi.org/10.1093/jac/37.6.1077
18. O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations. The Review on Antimicrobial Resistance, 2016. Retrieved from https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf
19. Otvos L. The short proline-rich antibacterial peptide family. Cell Mol Life Sci,2002:59(7):1138-1150. https://doi.org/10.1007/s00018-002-8493-8
20. Park SC, Park Y, Hahm KS. The role of antimicrobial peptides in preventing multidrug-resistant bacterial infections and biofilm formation. Int J Mol Sci,2011:12(9):5971-5992. https://doi.org/10.3390/ijms12095971
21. Powers JPS, Hancock REW. The relationship between peptide structure and antibacterial activity. Peptides,2003:24(11):1681-1691. https://doi.org/10.1016/j.peptides.2003.08.023
22. Reddy KVR, Yedery RD, Aranha C. Antimicrobial peptides: Premises and promises. Int J Antimicrob Agents,2004:24(6):536-547. https://doi.org/10.1016/j.ijantimicag.2004.09.005
23. Schägger H. Tricine–SDS-PAGE. Nat Protoc,2006:1(1):16-22. https://doi.org/10.1038/nprot.2006.4
24. Schuhmann B, Seitz V, Vilcinskas A, Podsiadlowski L. Cloning and expression of gallerimycin, an antifungal peptide expressed in immune response of greater wax moth larvae, Galleria mellonella. Arch Insect Biochem Physiol,2003:53(3):125-133. https://doi.org/10.1002/arch.10091
25. Simmaco M, Mignogna G, Barra D. Antimicrobial peptides from amphibian skin: What do they tell us? Biopolymers,1998:47(6):435-450. https://doi.org/10.1002/(SICI)1097-0282(1998)47:6<435::AID-BIP3>3.0.CO:2-8
26. Steiner H, Hultmark D, Engström Å, Bennich H, Boman HG. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature. 1981:292(5820):246-248. https://doi.org/10.1038/292246a0
27. *Vizi*oli J, Salzet M. Antimicrobial peptides from animals: Focus on invertebrates. Trends Pharmacol Sci,2002:23(11):494-496. https://doi.org/10.1016/S0165-6147(02)02105-3
28. Wang G, Li X, Wang Z. APD3: The antimicrobial peptide database as a tool for research and education.

Nucleic Acids Res,2016:44(D1):D1087-D1093. https://doi.org/10.1093/nar/gkv1278
29. WHO. Antimicrobial resistance: Global report on surveillance. World Health Organization, 2014. Retrieved from https://apps.who.int/iris/handle/10665/112642
30. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature,2002:415(6870):389-395. https://doi.org/10.1038/415389a