



Cecrosins from *Rhynchophorus ferrugineus* Gut: A Promising Peptide Antibiotic against Multiple Antibiotic-Resistant *Klebsiella pneumoniae* Strains

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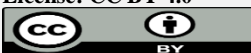
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Abstract	Article History
<p><i>Klebsiella pneumoniae</i>, a major pathogen, is increasingly resistant to antibiotics, posing a significant public health threat due to its role in various infections. This study aimed to isolate and characterize <i>K. pneumoniae</i> from clinical samples and evaluate the antibacterial activity of cecropins, antimicrobial peptides, against multidrug-resistant (MDR) <i>K. pneumoniae</i> isolates. <i>K. pneumoniae</i> isolates were characterized using cultural, morphological, biochemical, and molecular tests. Antibiotic resistance profiles were determined using standard methods. The antibacterial activity of cecropins was assessed using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Three <i>K. pneumoniae</i> strains A27782, K60365 and DD02425 (KPA2, KPK6, and KPDD) were identified, exhibiting extensive resistance to conventional antibiotics. Cecropins showed potent, strain-dependent bactericidal activity against the MDR isolates, with MIC and MBC values ranging from 0.00781 to 0.25000 mg/mL and 0.06250 to 0.50000 mg/mL, respectively. Statistical analysis confirmed significant differences in susceptibility among the isolates ($p < 0.05$). Cecropins exhibit promising antibacterial activity against MDR <i>K. pneumoniae</i> isolates, suggesting their potential as alternative therapeutic agents against antibiotic-resistant infections. This study highlights the potential of cecropins as novel antibacterial agents against MDR <i>K. pneumoniae</i>, providing a new avenue for combating antibiotic resistance.</p> <p>Keywords: <i>Klebsiella pneumoniae</i>, cecropins, antibiotic resistance, multidrug resistance, antibacterial activity</p>	<p>Received: 29 Dec 2025 Accepted: 14 Feb 2026 Published: 20 Feb 2026</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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Introduction

The rise of multidrug-resistant (MDR) Gram-negative pathogens represents one of the most pressing challenges to modern medicine, rendering many first- and last-line antibiotics ineffective (World Health Organization, 2021; Okeke *et al.*, 2017; Dim *et al.*, 2025a). *Klebsiella pneumoniae* is a preeminent threat within this group, frequently causing life-threatening hospital-acquired infections such as pneumonia, bacteremia, and urinary tract infections. Its propensity to rapidly acquire and disseminate resistance genes, including those encoding extended-spectrum beta-lactamases (ESBLs) and carbapenemases, has led to the global spread of pan-resistant clones with severely limited treatment options (Navon-Venezia *et al.*, 2017; Pitout *et al.*, 2015; Amadi *et al.*, 2017; Dim *et al.*, 2025b).

The transmission of these high-risk pathogens is facilitated by environmental reservoirs and mechanical vectors. The common housefly, *Musca domestica*, is a well-documented vector capable of mechanically transmitting *K. pneumoniae* and other resistant bacteria, particularly in and around healthcare facilities where it breeds in organic waste (Chaiwong *et al.*, 2014; Khamesipour *et al.*, 2018; Chude *et al.*, 2020; Dim *et al.*, 2025c). This epidemiological link underscores the critical need for innovative antimicrobials with novel mechanisms of action to disrupt the cycle of environmental transmission and clinical infection.

Antimicrobial peptides (AMPs) are essential components of the innate immune system across diverse organisms and represent a promising frontier in antibiotic discovery (Hancock

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& Sahl, 2006). Their cationic and amphipathic nature allows them to disrupt bacterial membranes, a mechanism associated with a lower propensity for resistance development compared to traditional, single-target antibiotics (Mahlapuu *et al.*, 2016). Insects, in particular, are a rich source of novel AMPs. The red palm weevil (*Rhynchophorus ferrugineus*), a devastating agricultural pest, harbors a unique gut microbiome that produces cecrosins—a class of potent AMPs originally identified for their role in host defense and symbiont regulation (Ding *et al.*, 2021; Shi *et al.*, 2022). While their activity against some plant pathogens has been characterized, their efficacy against clinically significant, multidrug-resistant human pathogens like *K. pneumoniae* remains largely unexplored.

Therefore, this study aimed to investigate the *in vitro* inhibitory and bactericidal efficacy of cecrosins against clinical, MAR strains of *K. pneumoniae* isolated from *M. domestica* in hospital landfill sites. By determining the minimum inhibitory and bactericidal concentrations, this research seeks to evaluate the potential of cecrosins as a novel therapeutic candidate in the urgent fight against antibiotic-resistant infections.

Materials and Methods

Sample Collection, Handling and Transportation: This was done using the method described in work published by Iheukwumere *et al.* (2025a), Iheukwumere *et al.* (2025b), Iheukwumere *et al.* (2025c), Egbe *et al.* (2025a). We used a sterilize spoon to gently scrape the debris from the soil surfaces. In the hospital dumping site, a sterile soil auger was driven down to a plough depth of 15 cm. Ten samples of soil were taken from each sampling unit and placed in a sterile tray. After carefully removing any extraneous items including roots, stones, pebbles, and gravel, the samples were properly combined. Next, the soil sample was quartered, reducing it to half. Once again, the soil sample was quartered by splitting it into four equal halves. The two opposing quarters were thrown away, and the other two quarters were combined. The other soil samples utilized in this investigation underwent the same procedure. After being properly labeled, the samples were stored in a sterile cooler to preserve the isolates' number and temperature. For analysis, the samples were brought to the lab. Twenty samples in all were gathered from various hospital disposal locations.

Culture and Isolation of Bacteria

An analytical weighing balance (JJ430BC) was employed to weigh 1 gram of the soil sample into a 50 mL Pyrex beaker. Three milliliters of normal saline (0.85% NaCl) were added, and the mixture was thoroughly shaken before being adjusted to a final volume of 10 mL with normal saline. A tenfold serial dilution was performed, and the sample was aseptically inoculated onto Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing MacConkey agar medium (MA/Biotech). All the plates in triplicates were incubated inverted at 37±2°C for 48 h as described by Egbe *et al.* (2025b), Egbe *et al.* (2025c), Iheukwumere *et al.* (2025d), Iheukwumere *et al.* (2025e).

Characterization and Identification of the Isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2°C for 24 h. The

isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al.* (2018b), Iheukwumere *et al.* (2025f), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020a), Iheukwumere *et al.* (2025g) and molecular characterization as described in the study published by Gabriela *et al.* (2014), Ekesiobi *et al.* (2025), Ekechukwu *et al.* (2025a), Ekechukwu *et al.* (2025b), Ezedianafu *et al.* (2025a), and Ezedianafu *et al.* (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al.* (2020b), Idigo *et al.* (2025a), Idigo *et al.* (2025b), Idigo *et al.* (2025c), Idigo *et al.* (2025d), and Ezedianafu *et al.* (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al.*, 2017; Iheukwumere *et al.*, 2017a; Iheukwumere *et al.*, 2017b; Iheukwumere *et al.*, 2023a; Iheukwumere *et al.*, 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al.* (2017c), Iheukwumere *et al.* (2018c) Ike *et al.* (2025a), Iheukwumere *et al.* (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35±2°C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al.* (2017d), Iheukwumere *et al.* (2022b), Iheukwumere *et al.* (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al.* (2025b) Ike *et al.* (2025c) Iheukwumere *et al.* (2022d), Idigo *et al.* (2025e), Obiefuna *et al.* (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al.* (2017), Obianom *et al.*

(2024), Ekechukwu *et al.* (2025c), Obiefuna *et al.* (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al.* (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37°C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025h), Ike *et al.* (2025d), Idigo *et al.* (2025e), Ezedianafo *et al.* (2025d), Ezedianafo *et al.* (2025e) and Iheukwumere *et al.* (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37°C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al.* (2025d), Ike *et al.* (2025e), Idigo *et al.* (2025f), Idigo *et al.* (2025g) and Obiefuna *et al.* (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45°C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37°C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al.* (2017), Iheukwumere *et al.* (2025j), Iheukwumere *et al.* (2025k), and Idigo *et al.* (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. The presence pink/red colouration indicated positive urease test

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al.* (2025h), Idigo *et al.* (2025i), Iheukwumere *et al.* (2025j) and Idigo *et al.* (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically

inoculated into the sterilized medium. This was incubated at 37°C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025j), Iheukwumere *et al.* (2025k), Idigo *et al.* (2025k), Idigo *et al.* (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α -naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al.* (2025c), and Idigo *et al.* (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37°C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al.* (2025l), Iheukwumere *et al.* (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al.* (2025c) Iheukwumere *et al.* (2025n), and Iheukwumere *et al.* (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al.*, 2025p; Iheukwumere *et al.*, 2025q; Chude *et al.*, 2020)

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with

a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al.*, 2025r; Iheukwumere *et al.*, 2025s; Ejike *et al.*, 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with $\geq 95\%$ similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al.*, 2017; Iheukwumere *et al.*, 2025t; Nwike *et al.*, 2017).

Susceptibility Patterns of the Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010). The isolates were aseptically subcultured into a broth culture and incubated at 35 ± 2°C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% BaCl₂ · 2H₂O and 99.5 mL of 1% Conc. H₂SO₄. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

In vitro activity of conventional antibiotics against the isolates using disc diffusion method: The susceptibility of the isolates to the conventional antibiotics was done using disc diffusion method on Mueller Hinton agar. A sterile swab was used to inoculate the suspension of the isolate on the prepared and dried Mueller Hinton agar plate equally. It was then left to stay for 5 minutes. A sterile forceps was used to place the commercially prepared antibacterial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 37°C for 24 h. Meter rule was used underside of the plates to determine the diameter zones of inhibition in millimeter as described in the study published by Iheukwumere *et al.* (2018).

Sample Collection, Handling, Transportation of Macrotermes species: *Rhynchophorus ferrugineus* samples were collected from decay raffia palms using hand picking and cleaned plastic containers. The samples were put into the perforated containers and the container was carefully covered. The covering of the containers deprived the larvae from oxygen resulting in death. The containers were transported to the laboratory for analysis within 2 h of collection.

Extraction of cecropins: Cecropins, a peptide antibiotic, were extracted from the gut of *Rhynchophorus ferrugineus* using a suitable solvent and thin layer chromatography (TLC). The process involved several steps. First, the guts of *Rhynchophorus ferrugineus* were dissected and homogenized in a phosphate-buffered saline (PBS) solution to release the cecropins peptide. The homogenate was then centrifuged to separate the supernatant, which contained the cecropins

peptide, from the cellular debris. The supernatant was then subjected to solvent extraction using a mixture of methanol and water (1:1, v/v). The methanol-water mixture was chosen as the solvent due to its ability to effectively solubilize the cecropins peptide. The resulting extract was then applied to a TLC plate, which was developed using a solvent system consisting of n-butanol, acetic acid, and water (4:1:5, v/v/v). The TLC plate was visualized under ultraviolet (UV) light, and the band corresponding to cecropins was identified based on its retention factor (R_f) value, which was approximately 0.55. The cecropins band was then scraped off the TLC plate and eluted with a small volume of methanol. The eluted cecropins were then concentrated and purified using high-performance liquid chromatography (HPLC).

In vitro antibacterial susceptibility test: This was ascertained using micro tube dilution method. Here, micro tube dilution plates was used. Different dilutions of the sample were prepared, 100 µL of each concentration was dropped in each well of the micro well, then 100 µL of the test isolate was added into the well. These were mixed and incubated at 37°C for 24 h. The bacterial growth pattern was determined for the most potent minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) as described by Clinical and Laboratory Standards Institute/CLSI (2015), Iheukwumere *et al.* (2025u), and Iheukwumere *et al.* (2025v).

Statistical Analysis: The results of the data generated were expressed as mean, percentage and Table, Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pair wise comparison of mean was done by Student “t” test as described in the study published by Iheukwumere *et al.* (2017e), Manasseh *et al.* (2025), Idigo *et al.* (2025n), Idigo *et al.* (2025o), Idigo *et al.* (2025p), Idigo *et al.* (2025q), Idigo *et al.* (2025r), Idigo *et al.* (2025s), Idigo *et al.* (2025t), Ugwu *et al.* (2025a) and Ugwu *et al.* (2025b).

Results

All three isolates (designated J, K, L) demonstrated identical cultural and biochemical characteristics typical of *Klebsiella pneumoniae*. Colonies on MacConkey agar were mucoid with a red to pink pigmentation, exhibiting a smooth surface, entire margin, and slightly raised elevation. Microscopic analysis confirmed Gram-negative, rod-shaped morphology. Biochemically, isolates were positive for catalase, citrate utilization, and the Voges-Proskauer test, and negative for oxidase, urease, hydrogen sulfide production, indole, and methyl red. 16S rRNA gene sequencing identified all isolates as *Klebsiella pneumoniae* with >99% sequence identity to reference strains. They were subsequently designated as follows: isolate J, *K. pneumoniae* strain A27782 (KPA2); isolate K, *K. pneumoniae* strain K60365 (KPK6); isolate L, *K. pneumoniae* strain DD02425 (KPDD). Among the total isolates collected, KPDD demonstrated the highest prevalence (45.10%), followed by KPK6 (37.25%) and KPA2 (17.65%).

All three isolates exhibited extensive resistance to conventional antibiotics, confirming their classification as multiple antibiotic-resistant (MAR) strains (Table 1). KPA2 and KPDD showed resistance to 8 out of 9 tested antibiotics (88.89% and 78.26% resistance rates, respectively), while KPK6 was resistant to 7 out

of 9 (77.78%). All isolates demonstrated concurrent resistance to ciprofloxacin (CP), streptomycin (S), penicillin (PN), cephalothin (CEP), trimethoprim-sulfamethoxazole (SXT), amoxicillin-clavulanate (AU), gentamicin (CN), and ofloxacin (ORF). The majority of isolates displayed a multiple antibiotic resistance (MAR) phenotype: 100% of KPA2 isolates, 88.89% of KPDD, and 76.92% of KPK6 were resistant to three or more classes of antibiotics.

The cecropin peptides exhibited potent, strain-dependent bactericidal activity against the MAR *K. pneumoniae* isolates (Table 2). Isolate KPK6 was the most susceptible, with a

minimum inhibitory concentration (MIC) of 0.00781 mg/mL and a minimum bactericidal concentration (MBC) of 0.06250 mg/mL at 24 hours. In contrast, isolate KPDD demonstrated the highest tolerance, requiring an MIC of 0.25000 mg/mL and an MBC of 0.50000 mg/mL for inhibition and killing, respectively. Isolate KPA2 showed intermediate susceptibility (MIC: 0.06250 mg/mL; MBC: 0.25000 mg/mL). Statistical analysis confirmed significant differences in susceptibility among the isolates ($p < 0.05$). The MBC/MIC ratios for all strains were ≤ 8 , confirming the primarily bactericidal mode of action of the cecropin peptides.

Table 1: Characteristics of the bacterial isolates

Characteristics	J	K	L
Appearance on MacConkey	Red/mucoid	Red/mucoid	Pink/mucoid
Elevation	Slightly raised	Slightly raised	Slightly raised
Surface edge	Smooth	Smooth	Smooth
Molility	-	-	-
Gram reaction	-	-	-
Cell morphology	Rods	Rods	Rods
Catalase	+	+	+
Oxidase	-	-	-
Urease	-	-	-
Citrate	+	+	+
Gelatin	+	+/-	+
Casein	+	+/-	+/-
H ₂ S	-	-	-
Indole	-	-	-
MR	-	-	-
VP	+	+	+
Glucose	+	+	+
Maltose	+	+	+
Xylose	+	+	+
Galactose	+/-	+/-	+
Inositol	+/-	+	+/-
Sorbitol	-	+/-	+/-
Citrate	+/-	+/-	+/-
Dulcitol	+/-	+/-	-

Table 2: Molecular characteristic of the isolates

Isolate code	Max score	Total score	Query cover (%)	E-value	Percent identity (%)	Accession Number	Description
J	1552	1552	100	0.0	100	CP181979	<i>Klebsiella pneumoniae</i> strain A27782 (KPA2) chromosome complete genome
K	2069	2069	100	0.0	100	CP168408	<i>Klebsiella pneumoniae</i> strain K60365 (KPK6)
L	1975	1975	100	0.0	100	CP189716	<i>Klebsiella pneumoniae</i> strain DD02425 (KPDD)

Table 3: Susceptibility of the isolates to conventional antibiotics

Isolate	N	Susceptible Strain (%)	Resistance Strain (%)	Implicated antibiotics
KPA2	9	1 (11.11)	8 (88.89)	S, S, PN, CH, SXT, AU, CN, ORF
KPK6	19	6 (31.58)	13 (68.42)	AMX, AU, CH, S, PN, SXT, CN
KPDD	23	5 (21.74)	18 (78.26)	PER, S, PN, CH, SXT, AU, CN, ORF
Total	51	12 (23.53)	39 (76.47)	

Table 4: Inhibitory activity of cecropins against the test isolates

Conc. (%)	KPA2	KPK6	KPDD
0.10	0.0000	0.0000	0.0000
0.20	0.0000	0.0000	0.0000
0.30	0.0000	0.0000	0.0000
0.40	0.0000	0.0000	0.0000
0.50	0.0000	0.0000	0.0000
0.60	0.0000	0.0000	0.0000
0.70	0.0000	0.0000	0.0000
0.80	0.5000	0.2500	0.5000
0.90	0.2500	0.1250	0.5000
1.00	0.1250	0.0625	0.2500

Discussion

This study provides evidence for the potent *in vitro* antibacterial activity of cecrosin peptides against clinically relevant, multidrug-resistant (MDR) strains of *Klebsiella pneumoniae*. The isolates, obtained from the high-risk environmental reservoir of *Musca domestica* in hospital landfill sites, exhibited extensive resistance, highlighting a critical public health challenge that cecrosins may help address.

The isolates, definitively identified as *K. pneumoniae* strains KPA2, KPK6, and KPDD, displayed classical phenotypic profiles consistent with previous reports (Ojo *et al.*, 2018). Their resistance profiles were particularly alarming, with KPA2 and KPDD demonstrating resistance to 88.89% and 78.26% of tested conventional antibiotics, respectively. This aligns with the global escalation of pan-drug resistance in *K. pneumoniae*, a major driver of hospital-acquired infection mortality and a priority pathogen for new drug development (World Health Organization, 2021; Pitout *et al.*, 2015). The high prevalence of multiple antibiotic resistance (MAR) phenotypes, especially the 100% rate in KPA2 isolates, underscores the urgent need for novel therapeutic agents with distinct mechanisms of action.

In this context, the significant bactericidal activity of cecrosins is a key finding. The observed strain-dependent efficacy, with KPK6 being the most susceptible (MIC: 0.00781 mg/mL) and KPDD the most tolerant (MIC: 0.25000 mg/mL), reflects the natural variability in membrane composition and defensive adaptations among clinical strains when exposed to cationic antimicrobial peptides (AMPs) (Hancock & Sahl, 2006). The MBC/MIC ratios of ≤ 8 for all strains confirm a primarily bactericidal mode of action, a desirable characteristic for treating severe systemic infections. This activity is consistent with the proposed mechanism of cecrosins and related AMPs, which involves electrostatic interaction with the negatively charged bacterial outer membrane followed by disruption of the cytoplasmic integrity—a target that poses a significant barrier to conventional resistance mechanisms (Mahlapuu *et al.*, 2016).

The source of these peptides, the gut of *Rhynchophorus ferrugineus*, represents an underexplored reservoir of bioactive compounds. The production of cecrosins by the weevil's gut symbionts for host defense and microbiome regulation (Ding *et al.*, 2021; Shi *et al.*, 2022) provides an evolutionary rationale for their potency. Our results successfully translate this ecological function into a potential

clinical application, demonstrating efficacy against highly resistant human pathogens sourced from a critical transmission vector.

The differential susceptibility between isolates (e.g., KPDD vs. KPK6) warrants further investigation. Potential factors include variations in lipopolysaccharide structure, outer membrane protein expression, or efflux pump activity, all of which can modulate interaction with cationic peptides. Future proteomic and transcriptomic analyses could elucidate the specific bacterial determinants influencing cecrosin susceptibility.

Conclusion

In conclusion, this study establishes that cecrosin peptides, derived from the gut microbiota of *Rhynchophorus ferrugineus*, exhibit potent *in vitro* bactericidal activity against environmentally sourced, multidrug-resistant *Klebsiella pneumoniae*. Their efficacy against strains with extensive resistance to conventional antibiotics positions them as promising lead candidates for the development of a novel class of peptide-based antimicrobials. Subsequent research should prioritize the large-scale synthesis and further purification of cecrosins, detailed mechanistic studies on their interaction with Gram-negative membranes, and rigorous evaluation of their pharmacodynamic and safety profiles in appropriate *in vivo* infection models.

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