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Moricins: A Potential Peptide Antibiotic from Silkworm against Antibiotic-Resistant *Klebsiella pneumoniae*

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Abstract

The rising threat of antibiotic-resistant *Klebsiella pneumoniae* has severely compromised treatment efficacy, necessitating innovative solutions. Moricins, peptide antibiotics from silkworm, show promise against *K. pneumoniae*, offering a potential alternative to combat antibiotic resistance and enhance therapeutic outcomes against this critical pathogen.This study aimed to isolate and characterize *K. pneumoniae* from clinical samples and evaluate the antibacterial activity of moricin, a peptide antibiotic from silkworm, against multidrug-resistant (MDR) *K. pneumoniae* isolates. *K. pneumoniae* isolates were characterized using cultural, morphological, biochemical, and molecular tests. Antibiotic resistance profiles were determined using standard methods. The antibacterial activity of moricin was assessed using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Three *K. pneumoniae* strains A27782, K60365 and DD02425 (KPA2, KPK6, and KPDD) were identified, exhibiting extensive multidrug resistance patterns. Moricin demonstrated significant antibacterial activity against all 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 revealed significant differences in susceptibility patterns among the isolates ($p < 0.05$). Moricin exhibits promising antibacterial activity against MDR *K. pneumoniae* isolates, suggesting its potential as an alternative therapeutic agent against antibiotic-resistant infections. This study highlights the potential of moricin as a novel antibacterial agent against MDR *K. pneumoniae*, providing a new avenue for combating antibiotic resistance.

Keywords: *Klebsiella pneumoniae*, moricin, antibiotic resistance, multidrug resistance, antibacterial activity

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Introduction

The relentless spread of multidrug-resistant (MDR) Gram-negative bacteria represents a formidable global health threat, drastically undermining the efficacy of existing antibiotics (World Health Organization, 2021; Okeke *et al*., 2017; Dim *et al*., 2025a). *Klebsiella pneumoniae* is a leading agent within this group, responsible for severe nosocomial infections including pneumonia, bloodstream infections, and complicated urinary tract infections. Its remarkable capacity to acquire and disseminate resistance genes, particularly those encoding extended-spectrum beta-lactamases (ESBLs) and carbapenemases, has led to the emergence of pan-resistant strains with few viable treatment options, resulting in increased morbidity and mortality (Bush & Bradford, 2020; Pitout *et al*., 2015; Amadi *et al*., 2017; Dim *et al*., 2025b).

The transmission cycle of these high-risk pathogens is often facilitated by environmental vectors. The common housefly, *Musca domestica*, is a proven mechanical vector for numerous enteric bacteria, including *K. pneumoniae*, especially in healthcare settings where it thrives on organic waste (Fotedar, 2001; Khamesipour *et al*., 2018; Chude *et al*., 2020; Dim *et al*., 2025c). This ecological link highlights the critical need for innovative antimicrobials with novel mechanisms of action to disrupt transmission and treat resistant infections.

Antimicrobial peptides (AMPs), key components of the innate immune system across diverse species, have emerged as a promising therapeutic avenue (Zasloff, 2002). Their typical cationic and amphipathic structure enables them to target and disrupt bacterial membranes, a mechanism associated with a lower risk of resistance development compared to conventional, single-target antibiotics (Mahlapuu *et al.*, 2016). Insects are a prolific source of such AMPs. The silkworm, *Bombyx mori*, produces a family of peptides known as moricins, which are induced upon immune challenge and exhibit broad-spectrum antimicrobial activity (Hara & Yamakawa, 1995; Li *et al.*, 2021). While their activity against some Gram-positive bacteria and fungi is documented, their efficacy against contemporary, clinically significant MDR Gram-negative pathogens like *K. pneumoniae* remains underexplored.

Therefore, this study aimed to investigate the *in vitro* inhibitory and bactericidal efficacy of moricin peptides 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 moricins as a novel therapeutic candidate in the urgent fight against antibiotic-resistant infections.

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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 (JJJ430BC) 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), Ezedianafo *et al*. (2025a), and Ezedianafo *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 Ezedianafo *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^{0}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 ≥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+ 2^0C 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% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. 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).

Extraction of moricins: Moricins, a peptide antibiotic, were extracted from the gut of silkworms using a suitable solvent and thin layer chromatography (TLC). The process involved several steps. First, the guts of silkworms were dissected and homogenized in a phosphate-buffered saline (PBS) solution to release the moricins peptide. The homogenate was then centrifuged to separate the supernatant, which contained the moricins 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 moricins 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 moricins was identified based on its retention factor (Rf) value, which was approximately 0.45. The moricins band was then scraped off the TLC plate and eluted with a small volume of methanol. The eluted moricins 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

Characterization and Prevalence of *Klebsiella pneumoniae* Isolates

The three *Klebsiella* species isolates (J, K, L) exhibited identical cultural, morphological, and biochemical profiles characteristic of *Klebsiella pneumoniae*. All isolates produced large, mucoid colonies with characteristic red to pink lactose fermentation on MacConkey agar, displaying a smooth surface, entire margin, and slightly raised elevation. Microscopic examination confirmed Gram-negative, rod-shaped morphology. Biochemically, isolates were positive for catalase, citrate utilization, and Voges-Proskauer tests, while negative for oxidase, urease, hydrogen sulfide production, indole, and methyl red. 16S rRNA gene sequencing definitively identified all isolates as *Klebsiella pneumoniae* with >99% sequence identity to reference strains. The isolates were designated as follows: Isolate J – *K. pneumoniae* strain A27782 (KPA2); Isolate K – *K. pneumoniae* strain K60365 (KPK6); Isolate L – *K. pneumoniae* strain DD02425 (KPDD). Analysis of prevalence revealed KPDD as the most common strain (45.10%), followed by KPK6 (37.25%) and KPA2 (17.65%).

Antibiotic Resistance Profiles of Clinical Isolates

All isolates demonstrated extensive multidrug resistance patterns consistent with their environmental hospital landfill origin. KPA2 exhibited resistance to 8 of 9 tested antibiotics (88.89%), KPK6 to 7 of 9 (77.78%), and KPDD to 8 of 9 (78.26%). All strains showed consistent high-level resistance to ciprofloxacin (CP), streptomycin (S), penicillin (PN), cephalothin (CEP), trimethoprim-sulfamethoxazole (SXT), amoxicillin-clavulanate (AU), and ofloxacin (ORF), with variable resistance to gentamicin (CN). A concerning 100% of KPA2 isolates, 88.89% of KPDD, and 76.92% of KPK6 displayed resistance to three or more antibiotic classes, confirming their classification as multiple antibiotic-resistant (MAR) strains.

In Vitro Efficacy of Moricin Peptides

The silkworm-derived antimicrobial peptide moricin demonstrated significant antibacterial activity against all MAR *K. pneumoniae* strains, with observable strain-dependent variation in susceptibility ($p < 0.05$). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays revealed KPK6 as the most susceptible strain (MIC: 0.00781 mg/mL; MBC: 0.06250 mg/mL), followed by KPA2 (MIC: 0.06250 mg/mL; MBC: 0.25000 mg/mL), with KPDD displaying the highest tolerance (MIC: 0.25000 mg/mL; MBC: 0.50000 mg/mL). The MBC/MIC ratios for all isolates ranged from 2 to 8, confirming moricin's primarily bactericidal mechanism of action against these resistant pathogens.

Statistical Analysis

Statistical analysis using one-way ANOVA confirmed significant differences in susceptibility patterns among the three *K. pneumoniae* isolates when exposed to moricin peptides ($F[2,15] = 28.47$, $p < 0.001$). Post-hoc Tukey tests revealed significant differences between KPK6 and KPDD ($p < 0.001$) and between KPA2 and KPDD ($p = 0.013$), but no significant difference between KPK6 and KPA2 ($p = 0.089$). These findings demonstrate that while all strains were susceptible to moricin, the degree of susceptibility varied significantly between strains, with KPDD requiring approximately 32 times higher concentration for inhibition compared to KPK6.

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	Slighyly raised
Surface edge	Smooth	Smooth	Smooth
Molility	-	-	-
Gram reaction	-	-	-
Cell morphology	Rods	Rods	Rods
Catalase	+	+	+
Oxidase	-	-	-
Urease	-	-	-
Citrate	+	+	+
Gelatin	+	+/-	+
Casein	+	+/-	+/-
H_2S	-	-	-
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	*Klebsiella pneumoniae* strain A27782 (KPA2) chromosome complete genome
K	2069	2069	100	0.0	100	CP168408	*Klebsiella pneumoniae* strain K60365 (KPK6)
L	1975	1975	100	0.0	100	CP189716	*Klebsiella pneumoniae* 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 cecrosins 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	0.5000	0.0000	0.0000
0.40	0.5000	0.0000	0.0000
0.50	0.5000	0.0000	0.0000
0.60	0.2500	0.5000	0.5000
0.70	0.1250	0.5000	0.2500
0.80	0.0625	0.2500	0.1250
0.90	0.0625	0.2500	0.1250
1.00	0.0625	0.2500	0.1250

Discussion

This study demonstrates the significant *in vitro* antibacterial efficacy of moricin, an antimicrobial peptide derived from the silkworm *Bombyx mori*, against clinical, multidrug-resistant (MDR) strains of *Klebsiella pneumoniae*. The high prevalence of resistance among the environmental isolates underscores the therapeutic potential of this insect-derived peptide in addressing a critical public health challenge.

The recovered isolates, definitively identified as *K. pneumoniae* strains KPA2, KPK6, and KPDD, displayed classical phenotypic profiles consistent with previous characterizations of this pathogen (Ojo et al., 2018). Their extensive resistance profiles are particularly alarming: KPA2 and KPDD exhibited resistance to 88.89% and 78.26% of tested conventional antibiotics, respectively. This aligns with global surveillance data documenting the rapid escalation of pan-drug resistance in *K. pneumoniae*, a priority pathogen for new drug development due to its significant contribution to hospital-acquired infection mortality (World Health Organization, 2021; Bush & Bradford, 2020). The near-ubiquitous presence of multiple antibiotic resistance (MAR) phenotypes, especially the 100% MAR rate in KPA2 isolates, highlights the diminishing utility of existing antibiotic classes and the urgent need for novel therapeutic agents with distinct mechanisms of action.

In this context, the potent bactericidal activity of moricin is a pivotal finding. The observed strain-dependent efficacy, with KPK6 being the most susceptible and KPDD the most tolerant, reflects the natural variability in bacterial membrane composition and defensive adaptations among clinical strains when challenged by cationic antimicrobial peptides (Hara & Yamakawa, 1995; Mahlapuu et al., 2016). The consistently low MBC/MIC ratios confirm a primarily bactericidal mechanism, a desirable trait for agents intended to treat serious systemic infections. This activity is consistent with the established mechanism of moricin and related cationic peptides, which involves initial electrostatic attraction to the negatively charged bacterial outer membrane followed by disruption of cytoplasmic integrity—a target that poses a significant barrier to conventional resistance mechanisms (Zasloff, 2002).

The source of this peptide, the silkworm, represents a well-established but still promising reservoir of bioactive compounds. Moricins are integral to the insect's humoral immune response, providing a robust evolutionary rationale for their potent, broad-spectrum activity (Li et al., 2021). Our results successfully extend this known activity to a highly relevant clinical context, demonstrating efficacy against contemporary, environmentally sourced MDR *K. pneumoniae* obtained from a recognized mechanical vector, *Musca domestica*.

The differential susceptibility between isolates (e.g., the 32-fold higher MIC for KPDD versus KPK6) warrants mechanistic investigation. Potential contributing factors include strain-specific variations in lipopolysaccharide structure, outer membrane protein composition, or the expression of efflux pumps that can expel cationic peptides. Future research should employ transcriptomic and proteomic approaches to delineate the specific bacterial adaptations that modulate moricin susceptibility.

Conclusion

In conclusion, this study establishes that moricin, a peptide derived from the silkworm *Bombyx mori*, exhibits potent *in vitro* bactericidal activity against environmentally sourced, multidrug-resistant *Klebsiella pneumoniae*. Its efficacy against strains demonstrating extensive resistance to conventional antibiotics positions it as a promising lead candidate for the development of a novel class of peptide-based antimicrobials. Subsequent research should prioritize large-scale production and further purification of moricin, detailed mechanistic studies on its interaction with Gram-negative membranes, and rigorous evaluation of its *in vivo* efficacy and safety profiles in appropriate infection models

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