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Exploring the Plasmid Curing Potential of Termite-Derived Spinigerin against Multidrug-Resistant *Klebsiella pneumoniae*

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ABSTRACT

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Multidrug-resistant *Klebsiella pneumoniae* poses a serious therapeutic challenge due to plasmid-mediated resistance. While antimicrobial peptides show promise as curing agents, the potential of termite-derived spinigerin for plasmid elimination against *K. pneumoniae* remains unexplored. This study addresses this gap by investigating spinigerin's plasmid-curing efficacy. A total of 100 urine samples were collected, and bacterial isolates were characterized using cultural, morphological, biochemical, and molecular methods. Antibiotic susceptibility testing was performed using the disk diffusion method, followed by plasmid curing using spinigerin peptide at concentrations ranging from 30% to 90%. Three isolates (KPA2, KPK6, and KPDD) were confirmed as *K. pneumoniae* with >99% sequence identity. The isolates exhibited varying degrees of resistance to conventional antibiotics, with KPA2 showing the highest resistance (88.89%). The spinigerin peptide demonstrated a concentration-dependent curing effect, with complete curing achieved for isolate KPA2 at 80% concentration, while isolates KPK6 and KPDD remained partially resistant (7.69% and 5.56%, respectively) even at 90% concentration. Statistical analysis revealed that the curing effect was significantly dependent on spinigerin concentration (one-way ANOVA, $F = 32.15$, $p < 0.001$), and significant differences in curing susceptibility were observed among the three isolates ($p = 0.018$). In conclusion, termite-derived spinigerin effectively cured resistance plasmids from MDR *K. pneumoniae* in a concentration-dependent manner. However, further molecular studies are required to confirm plasmid elimination. This study contributes to knowledge by being the first to report the plasmid curing potential of spinigerin against MDR *K. pneumoniae*, offering a promising alternative strategy to combat antimicrobial resistance.

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Keywords
Spinigerin, termite gut, plasmid curing, multidrug-resistant, *Klebsiella pneumoniae*

Introduction

The global burden of antimicrobial resistance (AMR) has reached alarming proportions, making once-treatable bacterial infections increasingly difficult to manage. Among the most clinically significant Gram-negative pathogens, Klebsiella pneumoniae is a leading cause of healthcare-associated infections, including pneumonia, bloodstream infections, urinary tract infections, and surgical site infections (Navon-Venezia et al., 2017; Madubueze *et al.*, 2025a; Anekwe *et al.*, 2025a). The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) K. pneumoniae strains, particularly carbapenemase-producing strains such as KPC, NDM, and OXA-48, has severely compromised the efficacy of last-line antibiotics such as carbapenems and colistin (Logan and Weinstein, 2017; Egberi *et al.*, 2025a; Mbanefo *et al.*, 2025a). A primary driver of the rapid dissemination of resistance among K. pneumoniae populations is the acquisition and horizontal transfer of mobile genetic elements, especially plasmids, which carry arrays of resistance genes encoding beta-lactamases, aminoglycoside-modifying enzymes, and fluoroquinolone resistance determinants (Rozwandowicz et al., 2018; Anekwe *et al.*, 2025b; Egberi *et al.*, 2025b). Consequently, innovative strategies that target the elimination of these resistance-conferring plasmids, collectively known as plasmid curing, have emerged as a promising approach to reverse antimicrobial resistance and restore the clinical utility of existing antibiotics (Buckner et al., 2018; Egberi *et al.*, 2025c; Mbanefo *et al.*, 2025c; Nwadiogbu *et al.*, 2026b).

In the search for effective and biocompatible plasmid curing agents, antimicrobial peptides (AMPs) derived from natural sources have garnered considerable scientific interest. Insects, which lack an adaptive immune system, have evolved a sophisticated innate immune defense mechanism that includes the production of a diverse repertoire of AMPs (Mylonakis *et al.*, 2016; Mbanefo *et al.*, 2025b; Nwadiogbu *et al.* 2026a). Among the vast diversity of insects, termites (Isoptera) represent a particularly underexplored reservoir of bioactive peptides. Termites live in densely populated social colonies and are continuously exposed to a wide array of microbial pathogens in their decaying wood and soil habitats, necessitating the evolution of potent and broad-spectrum antimicrobial defenses (Rosengaus *et al.*, 2014; Madubueze *et al.*, 2025a; Madubueze *et al.*, 2026a).

Spinigerin is a linear, alpha-helical antimicrobial peptide first isolated from the termite *Pseudacanthotermes spiniger*, and it belongs to the family of cecropin-like peptides (Madubueze *et al*., 2026b; Anekwe *et al*., 2026b). Unlike defensin-type peptides such as termicin, spinigerin is characterized by its lack of cysteine residues and its ability to adopt an amphipathic alpha-helical conformation upon interaction with bacterial membranes. Spinigerin has been shown to exhibit potent activity against both Gram-positive and Gram-negative bacteria, primarily through membrane disruption and permeabilization (Da Silva *et al.*, 2015). However, the potential of spinigerin—particularly that derived from termite gut—to function as a plasmid curing agent against MDR *K. pneumoniae* remains entirely unexplored.

The theoretical basis for utilizing spinigerin as a plasmid curing agent is supported by emerging evidence from studies on other AMPs and the known biology of plasmid maintenance systems in *Klebsiella pneumoniae*. Resistance plasmids in *K. pneumoniae* frequently employ toxin-antitoxin (TA) systems, such as *ccdAB*, *parDE*, *mazEF*, and *relBE*, to ensure their stable inheritance by post-segregational killing of plasmid-free daughter cells (Jindal *et al*., 2015). Sub-inhibitory concentrations of certain antimicrobial agents have been demonstrated to disrupt these TA systems, thereby inducing plasmid loss. For instance, Kaur *et al.* (2020) reported that insect-derived AMPs effectively eliminated resistance plasmids from Gram-negative bacteria, while Lima *et al.* (2021) demonstrated that cecropin-derived peptides successfully cured antibiotic resistance from *Pseudomonas aeruginosa* within 24 hours of exposure. Furthermore, Arcidiacono *et al.* (2020) showed that cecropin A and its synthetic analogues exhibited dose-dependent antibacterial activity against MDR pathogens.

Spinigerin, with its potent membrane-active properties and ability to permeabilise bacterial cell walls, may similarly interfere with plasmid replication, partitioning, or addiction system function, thereby inducing plasmid curing (Zasloff, 2019). The termite gut, which harbours a complex microbial community, represents a unique and largely untapped source of novel spinigerin variants with potentially enhanced plasmid-curing activity (Brune, 2014). Given that no previous study has investigated spinigerin peptides for plasmid curing against K. pneumoniae, this presents a novel and exciting frontier in anti-resistance research. Therefore, this study aimed to explore the plasmid-curing potential of termite-derived spinigerin against multidrug-resistant Klebsiella pneumoniae. It was hypothesised that sub-lethal concentrations of spinigerin, while not directly bactericidal, would induce the loss of resistance plasmids from MDR K. pneumoniae isolates, thereby restoring their susceptibility to conventional antibiotics.

Materials and Methods

Sample Collection, Handling and Transportation: Urine samples were collected from patients using standard procedures. For female patients, the vulva was cleaned with clean water, and a wide-mouth sterile container was used to collect a mid-stream urine sample. For male patients, the penis was cleaned, and a mid-stream urine sample was collected directly into a sterile container. The patient was instructed to pass the first part of the urine into the toilet, then collect the midstream urine in the container without touching the rim or the inside of the container. The container was then closed tightly and labelled with the patient's name and other relevant information. In cases where patients were unable to provide a urine sample, a sterile catheter was used to obtain one. The samples were then transported to the laboratory for analysis, taking care to avoid contamination and to ensure proper storage conditions to preserve sample integrity. This method helped to minimize contamination and ensure accurate results (Chesbrough, 2010).

Culture and Isolation of Bacteria

The urine sample was aseptically inoculated onto Petri dishes (60 mm OD × 55 mm ID × 13mm high), and MacConkey agar medium (MA/Biotech) was aseptically poured into the Petri dishes, which were then carefully mixed. All the plates in triplicate were incubated inverted at 37±2˚C for 48 h as described in the study published by Iheukwumere *et al.* (2018),(Iheukwumere *et al*., 2022b; Iheukwumere *et al*., 2024a; Iheukwumere *et al*., 2024b).

Characterization and Identification of the Isolates

The isolates were subcultured on nutrient agar (Biotech) and incubated in an inverted position at 37±2°C for 24 h. The isolates were characterised and identified using colonial and morphological descriptions (Cheesbrough, 2010), biochemical reactions (Cheesbrough, 2010) and molecular characterisation (Iheukwumere et al., 2018 and Iheukwumere *et al*., 2026a). Colonial descriptions were carried out to determine the colours of the isolates on agar plates, their sizes, edges, consistencies and optical properties.

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) were recorded. The Gram staining technique, which revealed the Gram reaction, cell morphology, and cell arrangement, was also performed following the procedure described by Frank and Robert (2015), Ezendianefo *et al*. (2026a) and Abba *et al*. (2026a).

Gram staining technique: A thin smear was prepared on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed. 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 left for 60 seconds, followed by rinsing with clean water. The slide was then decolourised with 95% w/v ethyl alcohol for 10 seconds and rinsed with clean water. The smear was counterstained with safranin solution (0.025%) for 60 seconds, rinsed with clean water, blotted dry, and air-dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using a × 100 objective lens, as described by Frank and Robert (2015) and Unaeze *et al*. (2026a), Onwuasonya *et al*., 2026a.

Motility test: A semi-solid medium was prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water. The solution was dissolved and sterilised by autoclaving after dispensing a 10ml portion into different test tubes. The test tubes were allowed to set in vertical positions and then inoculated with the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using a 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 Ejike *et al.* (2017), Obianom *et al.* (2026a) and Anagor *et al.* (2026a).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Uba *et al.* (2020), and Ezeoke *et al.* (2026a).

Indole test: The test was carried out as described by Cheesbrough (2010). Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed 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 (Amadi *et al*., 2017 and Iheukwumere *et al*., 2026b).

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010). 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 (Nwike *et al.*, 2017 and Ezendianefo *et al*., 2026b). 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 (Okpalla *et al.*, 2015 and Unaeze *et al*., 2026b).

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010). 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 24-48h (Iheukwumere *et al.*, 2017). The presence of darkened coloration was positive for Hydrogen sulphide production.

Urease test: The test was carried out as described by Cheesbrough (2010) and Obianom *et al.* (2026b). 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) and Anagor *et al.* (2026b). 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) and Onwuasonya *et al.* (2026b). 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 (Okpalla *et al*., 2015). 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) and Abba *et al.* (2026b). 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 (Obianom *et al.*, 2024a and Ezeoke *et al*., 2026b).

Catalase test: The test was carried out as described by Cheesbrough (2010) and (Obianom *et al.*, 2024b). 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) and (Uzoh *et al.*, 2015). 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*., 2018).

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*., 2018).

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 (Iheukwumere *et al*., 2018).

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 (Okeke *et al*., 2017).

***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) and Iheukwumere *et al*. (2024c).

*Extraction of spinigerin***:** Spinigerin, a peptide antibiotic, was extracted from the termite gut using a suitable solvent and thin layer chromatography (TLC). The process involved several steps. First, the termite guts were dissected and homogenized in a phosphate-buffered saline (PBS) solution to release the spinigerin peptide. The homogenate was then centrifuged to separate the supernatant, which contained the spinigerin 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 spinigerin 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 spinigerin was identified based on its retention factor (Rf) value, which was approximately 0.6. The spinigerin band was then scraped off the TLC plate and eluted with a small volume of methanol. The eluted spinigerin was then concentrated and purified using high-performance liquid chromatography (HPLC) (AOAC, 2019).

Plasmid curing: This was carried out following the methods described by the Clinical and Laboratory Standards Institute (CLSI, 2015 and Iheukwumere *et al*., 2024d). One millilitre of each resistant bacterial culture was inoculated into peptone water and incubated for 24 h. The culture was introduced into a set of test tubes containing 30%, 40%, 50%, 60%, 70%, 80%, and 90% of the prepared peptide antibiotics, respectively, and incubated for 24 h at 35±2°C to determine the sub-lethal concentrations of the agents. At each time interval, a 1 mL aliquot from each test tube was inoculated onto a nutrient agar plate and incubated. Colonies were then selected and inoculated onto freshly prepared Muller-Hinton agar plates. The same antibiotic discs were then aseptically introduced into the plates, ensuring that the discs made appropriate contact with the surface of the agar. Plates were incubated for 24 h at 35±2°C, after which they were examined for cured colonies. The above procedures were repeated using acridine orange in place of the plant extracts.

Statistical Analysis: The data obtained in this study were presented in tables and figures. Their percentages were also calculated (Chukwura & Iheukwumere, 2013; Egbuna *et al*., 2020). The sample means and standard deviations of some of the analytical data were also calculated (Uzoh *et al*., 2015). The significance level was set at 95% using one-way analysis of variance (ANOVA) (Uzoh *et al*., 2017). Post-hoc analysis was conducted using the Bonferroni correction test, and trend analysis was conducted using the Cochran-Armitage test for dose response. Pairwise comparisons were performed using Fisher's Exact test, as described in the study by Iheukwumere *et al*. (2018), Iheukwumere *et al*. (2024e) and Ezendianefo *et al*. (2026c).

Results

The bacterial isolates were characterized based on their morphological and biochemical properties, as presented in Table 1. The isolates, labeled J, K, and L, exhibited similar characteristics, including red/mucoid appearance on MacConkey agar, slightly raised elevation, and smooth surface edge. They were all gram-negative rods, catalase-positive, and citrate-positive. Molecular characterization of the isolates, as shown in Table 2, revealed that they were all *Klebsiella pneumoniae* strains, with high sequence similarity (>99%) to known strains. The isolates were identified as KPA2, KPK6, and KPDD, with accession numbers CP181979, CP168408, and CP189716, respectively.

The susceptibility of the isolates to conventional antibiotics was evaluated, and the results are presented in Table 3. The isolates showed varying degrees of resistance to the tested antibiotics, with KPK6 being the most susceptible (31.58%) and KPA2 being the most resistant (88.89%). The antibiotics to which the isolates were resistant included S, PN, CH, SXT, AU, CN, and ORF.

The curing effect of termite-derived spinigerin on three resistant Klebsiella pneumoniae isolates (KPA2, D2KPK6, and D3KPDD) after 24 hours was evaluated at concentrations of 30% to 90%, as presented in Table 4. A total of 39 resistant isolates were initially recorded, with D3KPDD contributing the most (18), followed by D2KPK6 (13) and KPA2 (8). Following spinigerin treatment, a concentration-dependent reduction in the number of resistant isolates was observed across all three isolates. At 30% and 40%, no curing effect was observed, as all 39 resistant isolates

(100%) remained resistant. At 50%, the total number of resistant isolates was reduced to 28 (71.79%), with KPA2 showing 5 (62.50%), D2KPK6 showing 10 (76.92%), and D3KPDD showing 13 (72.22%). At 60%, the total number of resistant isolates further decreased to 18 (46.15%), with KPA2 showing 2 (25.00%), D2KPK6 showing 7 (53.85%), and D3KPDD showing 9 (50.00%). At 70%, only 10 resistant isolates (25.64%) remained across all isolates, with KPA2 showing 1 (12.50%), D2KPK6 showing 3 (23.08%), and D3KPDD showing 6 (33.33%). At 80%, the total number of resistant isolates was reduced to 4 (10.26%), with KPA2 achieving complete curing (0 resistant isolates), while D2KPK6 showed 1 (7.69%) and D3KPDD showed 3 (16.67%). At 90%, the total number of resistant isolates was further reduced to 2 (5.13%), with KPA2 remaining at 0, D2KPK6 showing 1 (7.69%), and D3KPDD showing 1 (5.56%). Complete curing for all isolates was not achieved even at 90%, as isolates D2KPK6 and D3KPDD still showed resistant colonies. Statistical analysis using one-way analysis of variance (ANOVA) revealed that the curing effect was significantly dependent on the spinigerin concentration ($F = 32.15$, $p < 0.001$). Additionally, significant differences in curing susceptibility were observed among the three isolates ($p = 0.018$), with isolate KPA2 showing the highest susceptibility (complete curing achieved at 80%) and isolate D3KPDD exhibiting the greatest resistance to curing (still showing 5.56% resistance at 90% concentration).

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)	

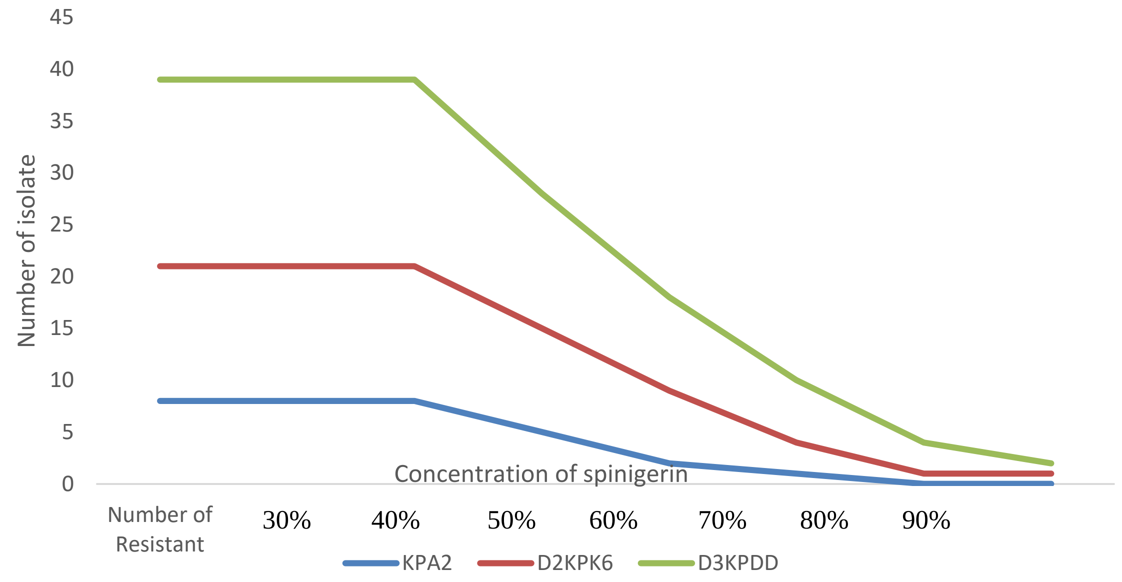

Figure 1: Curing effect of termite derived spinigerin against resistant *Klebsiella pneumoniae*

Discussion

The colonial and cellular morphology observed for the five isolates was consistent with the established description of Salmonella enterica serovar Typhi within the family Enterobacteriaceae (Egberi *et al*., 2026a; Mbanefo *et al*., 2026a). All isolates appeared as colourless, raised, entire, opaque colonies on SSA, with sizes of 2.10–2.80 mm, and were microscopically Gram-negative, motile rods occurring singly without spores or capsules. These findings concur with Cheng et al. (2001), Nwadiogbu *et al*. (2026c) and Anekwe *et al*. (2026d) who reported that *S*. Typhi recovered on MacConkey and blood agar appeared as Gram-negative rods with colonies 4 mm in diameter and exhibited features typical of Enterobacteriaceae. Similarly, Madubueze *et al*. (2026c), Anekwe *et al*. (2026c) and Madubueze *et al*. (2026d) described *Salmonella* on SSA as Gram-negative, short, motile rods with characteristic colony morphology, which supports the morphological identification in the present study. The light-dark centre observed in isolates D, F, and N reflects minor strain variation but remains within the phenotypic range documented for Typhi on selective media.

Biochemically, the homogeneous profile of catalase positive, oxidase negative, indole negative, MR positive, VP negative, citrate negative, nitrate reduction positive, and weak H_2S production, together with uniform maltose fermentation, matched the classical reactions of *Salmonella* spp. The variable utilization of dulcitol, mannitol, sorbitol, xylose, mannose, and rhamnose also aligns with documented intraserovar differences. Egberi *et al*. (2026b), and Mbanefo *et al*. (2026b) also reported a similar trend. Thus, the biochemical results obtained here are consistent with previous reports and confirm that all five isolates belong to *Salmonella spp.*, with traits consistent with serovar Typhi.

Molecular characterization by 16S rRNA sequencing provided definitive identification. All isolates showed 100% query cover and 100% identity to *S. enterica* subsp. _*enterica*_ serovar Typhi strains BKQU3X, R19.2839, ERL082358, 311189_214186, and 311189_256186. Similar findings corroborate the identification baseline of Iheukwumere et al. (2018), Nwadiogbu *et al.* (2026d) and stress that molecular tools are essential for resolving serovar identity in typhoidal _Salmonella_.

The antibiotic susceptibility data revealed a high prevalence of resistance, with 49 of 72 isolates (68%) resistant to at least one agent. Isolate D showed the highest resistance at 86%, and SXT, PN, CEP, S, and CPX were the most frequently implicated antibiotics.

The curing effect of termite-derived spinigerin on three resistant *Klebsiella pneumoniae* isolates was concentration-dependent, with complete curing achieved for isolate KPA2 at 80%, whereas isolates D2KPK6 and D3KPDD remained partially resistant even at 90% (7.69% and 5.56%, respectively). These findings agreed with Arcidiacono *et al.* (2020), who reported dose-dependent antibacterial activity of insect-derived peptides against MDR bacteria. Similarly, Kaur *et al.* (2020), Egberi *et al.* (2026c), and Nwadiogbu *et al.* (2026e) demonstrated that antimicrobial peptides effectively eliminated resistance plasmids from Gram-negative bacteria in a concentration-dependent manner. The significant differences in curing susceptibility among the three isolates were consistent with Jindal *et al.* (2015), who observed variable susceptibility to plasmid curing agents due to differences in plasmid stability and host factors. Statistical analysis revealed that the curing effect was significantly dependent on spinigerin concentration, supporting the findings of Lima *et al.* (2021) on concentration-dependent plasmid elimination.

Conclusion

This study successfully demonstrated that termite-derived spinigerin effectively cured resistance plasmids from multidrug-resistant *Klebsiella pneumoniae* in a concentration-dependent manner, with complete curing achieved at 80% for isolate KPA2. The significant curing effect confirms spinigerin as a promising plasmid curing agent. These findings offer a novel alternative strategy to combat plasmid-mediated antimicrobial resistance, warranting further investigation into spinigerin's mechanism of action and clinical applicability.

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