RESEARCH

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Combating Multidrug-Resistant *Pseudomonas aeruginosa*: Plasmid Curing Potentials of Novel Termicin Peptides from Termite Gut

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ABSTRACT

The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* has become a critical public health threat, necessitating novel therapeutic strategies such as plasmid curing. This study aimed to investigate the plasmid-curing potentials of novel termicin peptides extracted from termite gut against MDR *P. aeruginosa* isolated from fish pond water samples in Uli community, Anambra State, Nigeria. A total of 100 water 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 termicin peptide at concentrations ranging from 30% to 90%. Three isolates (PA03, PA065, and PA076) were confirmed as *P. aeruginosa* with >99% sequence identity. The isolates exhibited a multidrug resistance rate of 82.35% and an overall resistance rate of 43.59%. The termicin peptide demonstrated a concentration-dependent curing effect, with complete curing achieved for isolate PA076 at 80% concentration, while isolate PA065 remained partially resistant (7.14%) even at 90%. Statistical analysis revealed that the curing effect was significantly dependent on termicin concentration (one-way ANOVA, $F = 28.47$, $p < 0.001$), and significant differences in curing susceptibility were observed among the three isolates ($p = 0.021$). Therefore, termicin peptides from termite gut effectively cured resistance plasmids from MDR *P. aeruginosa* 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 termite gut-derived termicin peptides against MDR *P. aeruginosa*, offering a promising alternative strategy to combat antimicrobial resistance.

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Keywords
Termicin, termite gut, plasmid curing, multidrug-resistant, *Pseudomonas aeruginosa*

Introduction

The escalating crisis of antimicrobial resistance (AMR) has emerged as one of the most formidable challenges to global public health in the 21st century. Among the most concerning pathogens is *Pseudomonas aeruginosa*, a Gram-negative, opportunistic bacterium responsible for a wide range of healthcare-associated infections, including ventilator-associated pneumonia, bloodstream infections, urinary tract infections, and wound infections in burn victims and immunocompromised patients (Gellatly & Hancock, 2013; Madubueze et al., 2025a; Anekwe et al., 2025a). The rising prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. aeruginosa* strains has severely compromised the efficacy of conventional antibiotic therapies, leaving clinicians with limited or no treatment options (Bassetti et al., 2018; Egberi et al., 2025a; Mbanefo et al., 2025a).

In the quest for novel plasmid-curing agents, antimicrobial peptides (AMPs) derived from natural sources have shown remarkable promise. Insects, which lack an adaptive immune system, depend heavily on a robust innate immune response that includes the production of a diverse arsenal of AMPs (Mylonakis et al., 2016; Anekwe et al., 2025b; Egberi et al., 2025b). Among the vast diversity of insects, termites (Isoptera) represent an underexplored reservoir of bioactive peptides. Termites inhabit complex social colonies and are constantly exposed to diverse microbial pathogens in their decaying wood and soil environments, necessitating the evolution of potent antimicrobial defenses (Rosengaus et al., 2014; Mbanefo et al., 2025b; Nwadiogbu et al., 2026a). Termicin is a cysteine-rich, antifungal and antibacterial peptide first isolated from the termite *Pseudacanthotermes spiniger*, and it belongs to the defensin family of AMPs. Unlike cecropins, termicins are characterized by their stabilized beta-sheet structure and have been shown to exhibit broad-spectrum activity against both Gram-positive and Gram-negative bacteria (Madubueze et al., 2026b; Anekwe et al., 2026b). However, the potential of termicin peptides—particularly those derived from the termite gut microbiota—to function as plasmid curing agents against MDR *P. aeruginosa* remains entirely unexplored (Egberi et al., 2025c; Mbanefo et al., 2025c; Nwadiogbu et al., 2026b).

The theoretical basis for using termicin peptides as plasmid-curing agents is supported by emerging evidence from studies of other AMPs and by the known biology of plasmid maintenance systems. Plasmids harboured by *P.*

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aeruginosa often employ toxin-antitoxin (TA) systems, such as *ccdAB*, *parDE*, and *higBA*, 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 *P. 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 *P. aeruginosa*. Termicin, with its membrane-active properties and ability to permeabilize bacterial cell walls, may similarly interfere with plasmid replication, partitioning, or addiction system function (Zasloff, 2019). The termite gut, which harbours a complex microbial community, represents a unique and largely untapped source of novel termicin variants with potentially enhanced plasmid curing activity (Brune, 2014). Given that no previous study has investigated termicin peptides for plasmid curing, this presents a novel and exciting frontier in anti-resistance research (Madubueze et al., 2026a; Anekwe et al., 2026a).

Therefore, this study aimed to investigate the plasmid-curing potential of novel termicin peptides derived from termite gut against multidrug-resistant *Pseudomonas aeruginosa* (Nwadiogbu et al., 2025a; Madubueze et al., 2026b; Anekwe et al., 2026b). It was hypothesized that sub-lethal concentrations of these termicin peptides, while not directly bactericidal, would induce the loss of resistance plasmids from MDR *P. aeruginosa* isolates, thereby restoring their susceptibility to conventional antibiotics.

Materials and Methods

Sample Collection of water samples: Sample collection, handling and transportation: The samples used for this study were drawn from the fish pond. A total of 100 fish pond water samples were collected from five different locations in Uli community. The fish pond water samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and finally rinsed three times with distilled water (Iheukwumere *et al.*, 2018). The containers were placed inverted to drain the water inside. The container was inverted, lowered 5 cm below the fish pond water sample, then placed vertically to allow the water sample to refill the container. This sample was covered immediately, kept in a cooler with an ice block, and transported to the laboratory for immediate analysis (Iheukwumere *et al*., 2020).

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 24-48 h (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

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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 termicin: Termicin, 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 termicin peptide. The homogenate was then centrifuged to separate the supernatant, which contained the termicin 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 termicin 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 termicin was identified based on its retention factor (Rf = 0.40 - 0.50) value. The termicin band was then scraped off the TLC plate and eluted with a small volume of methanol. The eluted termicin 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 cultural and morphological characteristics of the isolates D1, D2, and D3 are presented in Table 1. The isolates exhibited similar characteristics, including blue-green appearance on Cetrimide agar, smooth edges, and rod-shaped cells. They were all motile, Gram-negative, and catalase-positive. The isolates were also positive for cetrimide and citrate tests, but negative for indole, methyl red, and Voges Proskauer tests. Molecular analysis of the isolates, presented in Table 2, confirmed their identity as *Pseudomonas aeruginosa* strains (LG03, F065, F076), with high sequence identity (>99%) to known strains. The Max score, Total score, and E-value indicated significant matches, with percent identity ranging from 100%.

The susceptibility of the isolates to conventional antibiotics is presented in Table 3. The overall susceptibility rate was 56.41%, with 43.59% of the isolates resistant to the tested antibiotics. The isolates exhibited varying degrees of resistance to antibiotics such as AMX, AU, PN, CEP, SXT, and CN. Statistical analysis revealed significant differences in resistance patterns among the isolates ($p < 0.05$). The degree of resistance exhibited by the isolates is presented in Table 4. The majority of the isolates (82.35%) exhibited multi-antibiotic resistance, with 17.65% resistant to a single antibiotic. This suggests that the isolates have developed complex resistance mechanisms, making them challenging to treat.

The curing effect of termicin on three resistant *Pseudomonas aeruginosa* isolates (PA03, PA065, and PA076) after 24 hours was evaluated across varying concentrations ranging from 30% to 90%, as presented in Table 5. A total of 34 resistant isolates were initially recorded across all isolates, with isolate PA065 contributing the highest number (14 resistant isolates), followed by PA03 (12 resistant isolates) and PA076 (8 resistant isolates). Following treatment with termicin, a concentration-dependent reduction in the number of resistant isolates was observed across all three isolates. At 30% and 40% concentrations, no curing effect was observed, as all 34 resistant isolates (100%) remained resistant. At 50% concentration, the total number of resistant isolates was reduced to 25 (73.53%), with isolate PA03 showing 8 resistant isolates (23.53%), PA065 showing 10 (29.41%), and PA076 showing 7 (20.59%). At 60% concentration, the total number of resistant isolates further decreased to 18 (52.94%), with PA03 and PA076 both showing 6 (17.65%) and 4 (17.65%) resistant isolates, respectively, while PA065 showed 8 (23.53%). At 70% concentration, only 9 resistant isolates (26.00%) remained across all isolates, with PA03 and

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PA076 showing 3 (8.82%) and 2 (8.82%) respectively, while PA065 showed 4 (11.76%). At 80% concentration, the total number of resistant isolates was reduced to 3 (8.82%), with PA03 showing 1 (2.94%) and PA065 showing 2 (5.88%), while PA076 achieved complete curing (0 resistant isolates). Complete curing for all isolates was not achieved even at 90% concentration, as isolate PA065 still showed 1 resistant isolate (2.94%). Statistical analysis using one-way analysis of variance (ANOVA) revealed that the curing effect was significantly dependent on the termicin concentration ($F = 28.47$, $p < 0.001$). Additionally, significant differences in curing susceptibility were observed among the three isolates ($p = 0.021$), with isolate PA076 showing the highest susceptibility (complete curing achieved at 80%) and isolate PA065 exhibiting the greatest resistance to curing (still showing 7.14% resistance at 90% concentration).

Table 1: Cultural and Morphological Characteristics of the Isolates

Parameter	D1	D2	D3
Appearance on Cetrimide agar	Blue-green	Colourless	Blue-green
Appearance on Nutrient agar	Blur-green	Bluish	Blue-green
Edge	Smooth	Smooth	Smooth
Surface	Smooth	Smooth	Smooth
Motility	+	+	+
Gram Reaction	-	-	-
Cell morphology	Rods	Rods	Rods
Catalase	+	+	+
Cetrimide test	+	+	+
Citrate	+	+	+
Indole	-	-	-
Methyl red	-	-	-
Voges Proskauer	-	-	-
Oxidase	+	+_	+
Glucose	-	-	-
Maltose	-	-	-
Fructose	+	+/-	+/-
Galactose	-	-	-
Inositol	-	-	-
Xylitol	-	+/-	-

Table 2: Molecular characteristics of the bacterial isolates

Isolate code	Max score	Toal score	Query cover (%)	E-value	Percent identity (%)	Accession Number	Description
D1	1672	1672	100	0.0	100	CP129520.1	*Pseudomonas aeruginosa* strain LG03 (PA03)
D2	1821	1821	100	0.0	100	CP115810.1	*Pseudomonas aeruginosa* strain F065 (PA065)
D3	1692	1692	100	0.0	100	CP115198.1	*Pseudomonas aeruginosa* strain F076 (PA076)

Table 3: Susceptibility of the isolates to conventional antibiotics

Isolate	Number	Susceptible Strain (%)	Resistant strain (%)	Implicated Antibiotic
PA03	26	14(53.85)	12(46.35)	AMX, AU, PN, CEP, SXT, CN
PA065	36	22(61.11)	14(38.89)	AMX, S, PN, SXT, CEP.
PA076	16	8(50.00)	8(50.00)	AU, AMX, S, PN, SXT, CEP
Total	78	44(56.41)	34(43.59)	

Table 4: Degree of resistance exhibited by the isolates

Isolate	Number of resistant strain	Single antibiotic resistant strain (%)	Multiantibiotic resistant strain (%)
PA03	12	3(25.00)	9(75.00)
PA065	14	2(14.29)	12(85.71)
PA076	8	1(12.50)	7(87.50)
Total	34	6(17.65)	28(82.35)

Table 5: Curing effect of termicin on the resistant isolates after 24 h

Isolate Code	Number of Resistant	3%	4%	5%	6%	7%	8%	9%
PA03	12 (35.29)	12(35.29)	12(35.29)	8(23.53)	6(17.65)	3 (8.82)	1 (2.94)	0 (0.00)
PA065	14 (41.18)	14(41.18)	14(41.18)	10(29.41)	8(23.53)	4(11.76)	2(5.88)	1(2.94)
PA076	8 (23.53)	8(23.53)	8(23.53)	7(20.59)	4(11.76)	2(5.88)	0 (0.00)	0 (0.00)
Total	34 (100.00)	34(100.00)	34(100.00)	25(73.53)	18(52.94)	9(26.00)	3(8.82)	1(2.94)

Discussion

The cultural and morphological characteristics of the isolates D1/PA03, D2PA065, and D3/PA076 were consistent with previous reports on *Pseudomonas aeruginosa* (Egberi *et al*., 2026a; Mbanefo *et al*., 2026a). The blue-green appearance on Cetrimide agar and rod-shaped cells are characteristic features of this bacterium. Molecular analysis confirmed the isolates as *Pseudomonas aeruginosa* strains (LG03, F065, F076), with high sequence identity (>99%) to known strains (Livermore *et al.*, 2012; Nwadiogbu *et al*., 2026c and Anekwe *et al*., 2026d). The Max score, Total score, and E-value indicated significant matches, with percent identity ranging from 100%.

The antibiotic susceptibility patterns of the isolates revealed varying degrees of resistance, with an overall susceptibility rate of 56.41% (Table 3). This is concerning, as *Pseudomonas aeruginosa* is known to exhibit multi-drug resistance (Gellatly *et al.*, 2013; Reynolds and Kollef, 2021; Madubueze *et al*., 2026c). The isolates exhibited resistance to multiple antibiotics, including AMX, AU, PN, CEP, SXT, and CN, highlighting the need for alternative therapeutic strategies (Wu *et al*., 2018; Anekwe *et al*., 2026c; and Madubueze *et al*., 2026d). The degree of resistance exhibited by the isolates was alarming, with 82.35% exhibiting multi-antibiotic resistance (Table 4). This is consistent with previous reports on the increasing prevalence of multidrug-resistant *Pseudomonas aeruginosa* strains (Mahlapuu *et al*., 2020; Pang *et al.*, 2019).

The curing effect of termicin on three resistant *Pseudomonas aeruginosa* isolates (PA03, PA065, and PA076) was found to be concentration-dependent, with complete curing achieved for isolate PA076 at 80% concentration, while PA065 remained partially resistant even at 90% (7.14%). These findings agreed with Arcidiacono *et al.* (2020), Egberi *et al*. (2026b), and Mbanefo *et al*. (2026b), who reported dose-dependent antibacterial activity of insect-derived peptides against MDR *P. aeruginosa*. Similarly, Kaur *et al.* (2020) 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), Nwadiogbu *et al*. (2026d), Egberi *et al*. (2026c), 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 termicin concentration supporting the findings of Lima *et al.* (2021) and Nwadiogbu *et al*. (2026e) on concentration-dependent plasmid elimination.

Conclusion

This study conclusively demonstrated that termite gut-derived termicin peptides effectively cured resistance plasmids from multidrug-resistant *Pseudomonas aeruginosa* in a concentration-dependent manner, achieving complete curing for isolate PA076 at 80% concentration. The significant concentration-dependent effect confirms termicin as a promising plasmid curing agent. These findings provide foundational evidence for termicin as a novel alternative strategy to combat antimicrobial resistance.

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