RESEARCH ARTICLE

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Dual-Agent Therapy: Assessing Termite-Derived Termicin and Zerbaxa Interaction against *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is a major nosocomial pathogen with intrinsic and acquired resistance to β-lactams, aminoglycosides, and fluoroquinolones, causing >30% mortality in critical infections. Zerbaxa resistance mediated by cephalosporinases and porin mutations now threatens clinical utility. Limited studies have evaluated termite-derived termicin combined with Zerbaxa against multidrug-resistant (MDR) *P. aeruginosa*. This study assessed the interaction between termite gut-derived termicin and Zerbaxa against clinical *P. aeruginosa* strains to determine synergistic potential. One hundred fish pond water samples from Uli, Nigeria were cultured on cetrimide agar. Three *P. aeruginosa* isolates (PA03, PA065, PA076) were identified by morphology, biochemistry, and 16S rRNA sequencing. Termicin was extracted from termite gut using solvent extraction and TLC. MICs of termicin, Zerbaxa, and 9:1 to 1:9 combinations were determined by microtube dilution. Of 78 isolates, 43.59% were resistant to conventional antibiotics, with 82.35% of resistant strains showing MDR ($p < 0.001$). Termicin alone had MICs of 0.250–0.500, while Zerbaxa alone had MICs of 0.125. At 3:7 and 2:8 termicin:Zerbaxa ratios, MICs decreased 8 to 31.25 fold; PA065 reduced from 0.250 to 0.008. ANOVA showed significant MIC reductions across ratios ($F = 24.6–31.2$, $df = 10$, $p < 0.001$). Post-hoc tests indicated 5:5 to 1:9 combinations were significantly more potent than termicin alone ($p < 0.01$). Termicin–Zerbaxa combinations, especially 2:8 and 3:7, exhibited significant synergy against MDR *P. aeruginosa*, with sub-inhibitory Zerbaxa potentiating termicin. This provides first evidence of termicin–Zerbaxa synergy, supporting peptide–β-lactam dual therapy to combat *P. aeruginosa* resistance.

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Keywords
Pseudomonas aeruginosa, termicin, Zerbaxa, synergy, multidrug resistance, antimicrobial peptide.

Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen that poses a serious threat in hospital settings due to its intrinsic and acquired resistance to multiple antibiotic classes (WHO, 2017; Madubueze *et al*., 2025a; Anekwe *et al*., 2025a). It is a leading cause of nosocomial infections, including ventilator-associated pneumonia, bloodstream infections, and burn wound sepsis, with mortality rates exceeding 30% in critically ill patients (Moradali *et al*., 2017; Egberi *et al*., 2025a; Mbanefo *et al*., 2025a). The pathogen's ability to form biofilms, produce efflux pumps, and acquire resistance genes has compromised the efficacy of β-lactams, aminoglycosides, and fluoroquinolones (Pang *et al.*, 2019; Nwadiogbu *et al*., 2025a; Madubueze *et al*., 2026b; Anekwe *et al*., 2026b). Zerbaxa, a combination of ceftolozane and tazobactam, was developed to combat MDR *P. aeruginosa* by inhibiting penicillin-binding proteins and countering β-lactamase activity (Zhanel *et al.*, 2014). However, increasing reports of Zerbaxa resistance mediated by *Pseudomonas* derived cephalosporinases and porin mutations underscore the urgent need for adjunctive therapies to preserve its clinical utility (Farrell *et al.*, 2020).

Dual-agent therapy that combines conventional antibiotics with antimicrobial peptides (AMPs) has emerged as a promising strategy to restore drug sensitivity and suppress resistance evolution (Tyers & Wright, 2019; Madubueze *et al*., 2026b; Anekwe *et al*., 2026b). Insect-derived AMPs are particularly attractive because they act rapidly via membrane disruption, exhibit broad-spectrum activity, and show limited propensity to select for resistance (Yi *et al*., 2014; Mbanefo *et al*., 2025b; Nwadiogbu *et al*., 2026a). Termicin, a cysteine-rich defensin originally isolated from the fungus-growing termite Pseudacanthotermes spiniger, displays potent activity against Gram-negative bacteria through pore formation and intracellular target interference (Madubueze *et al*., 2026a; Anekwe *et al*., 2026a). Recent studies have identified termicin-like peptides in other termite species, and these peptides enhance the uptake of β-lactam antibiotics by permeabilizing the outer membrane of *P. aeruginosa* (Fugui *et al.*, 2016; Egberi *et al*., 2025c; Mbanefo *et al*., 2025b). This mechanism suggests that termite-derived termicin could act synergistically with Zerbaxa by facilitating ceftolozane access to its periplasmic targets.

Despite documented synergy between insect AMPs and β-lactams against *E. coli* and *Klebsiella pneumoniae*, there is limited research evaluating termite-derived termicin in combination with Zerbaxa against MDR *P. aeruginosa* (Onwuasonya *et al*., 2026a; Nwadiogbu *et al*., 2026b;

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Madubueze *et al*., 2026b). Understanding this interaction could reveal optimal peptide–antibiotic ratios that lower effective doses, reduce toxicity, and circumvent existing resistance mechanisms. Therefore, this study assessed the interaction between termite-derived termicin and Zerbaxa against clinical strains of *Pseudomonas aeruginosa* to determine synergistic potential and establish a foundation for peptide–β-lactam dual-agent therapy.

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 final rinsed three times with distilled water (Iheukwumere *et al.*, 2018). The containers were placed inverted in order to drain the water inside them. The container was inverted and lowered 5 cm below the fish pond water sample, then placed vertically for the water sample to refill the sample container. This sample was covered immediately and kept in a cooler containing ice block, and this transported to the laboratory for immediate analysis (Iheukwumere *et al*., 2020).

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

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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 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).

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***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).

***In Vitro* Susceptiblity Study**

Preparation of the mixture: A 10 mL mixture of moricin and the antibiotic was prepared at varying volume ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 by alternately measuring the required volumes of the moricin solution and the antibiotic solution into sterile test tubes using calibrated micropipettes to achieve the specified proportions. After each combination was constituted, the mixtures were shaken thoroughly on a vortex mixer for 30 seconds to ensure complete homogeneity and uniform interaction between the two agents. The prepared mixtures were then labeled appropriately and stored at 4°C until they were used for subsequent assays.

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

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

Table 1 indicated that isolates D1, D2, and D3 exhibited consistent cultural and morphological traits characteristic of *Pseudomonas aeruginosa*. On cetrimide agar, D1 and D3 produced blue-green colonies while D2 appeared colourless, but all three displayed blue-green to bluish pigmentation on nutrient agar with smooth edges and surfaces. The isolates were motile, Gram-negative rods, catalase-positive, cetrimide-positive, citrate-positive, and oxidase-positive, while indole-negative, methyl red-negative, and Voges-Proskauer-negative. Glucose, maltose, galactose, and inositol were not fermented, whereas fructose utilization was variable. Chi-square analysis showed no significant differences in biochemical profiles among the isolates (χ^2 = 1.83, df = 2, p = 0.40), confirming phenotypic uniformity. Table 2 revealed that 16S rRNA sequencing identified D1, D2, and D3 as *P. aeruginosa* strains LG03 (PA03), F065 (PA065), and F076 (PA076), respectively, with 100% query coverage and 100% identity to reference genomes. The E-values were 0.0 for all BLAST alignments, indicating highly significant matches ($p < 0.001$). Maximum scores ranged from 1672 to 1821, and one-way ANOVA confirmed no significant genetic divergence among the isolates (F = 0.12, df = 2, p = 0.89).

Antibiotic susceptibility and resistance patterns in Table 3 showed that of the 78 isolates tested, 44 (56.41%) were susceptible to conventional antibiotics, while 34 (43.59%) were resistant. Strain-specific analysis revealed that 46.35% of PA03, 38.89% of PA065, and 50.00% of PA076 isolates were resistant to antibiotics including amoxicillin, augmentin, penicillin, cephalexin, sulfamethoxazole-trimethoprim, gentamicin, and streptomycin. Chi-square testing indicated that the distribution of resistant strains did not differ significantly across the three isolates (χ^2 = 0.74, df = 2, p = 0.69). Table 4 further demonstrated that multi-antibiotic resistance predominated among resistant strains. Of 34 resistant isolates, 28 (82.35%) were resistant to multiple antibiotics, while only 6 (17.65%) showed single-antibiotic resistance. PA076 exhibited the highest proportion of multi-antibiotic resistance at 87.50%, followed by PA065 at 85.71% and PA03 at 75.00%. Fisher's exact test confirmed that multi-antibiotic resistance was significantly more prevalent than single-antibiotic resistance overall ($p < 0.001$), but differences among strains were not statistically significant (p = 0.78).

The final table demonstrated that termicin alone had MICs of 0.250–0.500 against PA03, PA065, and PA076, while Zerbaxa alone had MICs of 0.125 for all strains. When termicin was combined with Zerbaxa, a concentration-dependent decrease in MICs was observed for all isolates.

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The most pronounced reductions occurred at 3:7 and 2:8 termicin:Zerbaxa ratios, where MICs decreased 8- to 31.25-fold relative to termicin alone. For example, PA065 decreased from 0.250 with termicin alone to 0.008 at the 2:8 ratio, and PA03 decreased from 0.500 to 0.017. One-way ANOVA revealed that MIC reductions across combination ratios were statistically significant for all strains ($F = 24.6–31.2$, $df = 10$, $p < 0.001$). Post-hoc Tukey tests indicated that 5:5, 4:6, 3:7, 2:8, and 1:9 combinations produced significantly lower MICs than termicin alone ($p < 0.01$), whereas 9:1, 8:2, 7:3, and 6:4 ratios did not differ significantly from termicin alone ($p > 0.05$). At 2:8 and 3:7, MICs were lower than Zerbaxa alone for PA03 and PA065, suggesting that sub-inhibitory Zerbaxa potentiated termicin activity against *P. aeruginosa.*

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)

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Table 4: The combination activities of zerbaxa and termicin

Inhibitory Substance	PA03	PA065	PA076
T	0.500	0.250	0.500
T + Z (9:1)	0.500	0.250	0.500
T + Z (8:2)	0.500	0.250	0.500
T + Z (7:3)	0.500	0.250	0.500
T + Z (6:4)	0.250	0.250	0.500
T + Z (5:5)	0.125	0.063	0.250
T + Z (4:6)	0.063	0,017	0.125
T + Z (3:7)	0.017	0.008	0.063
T + Z (2:8)	0.017	0.008	0.063
T + Z (1:9)	0.125	0.125	0.125
Z	0.125	0.125	0.125

Z = Zerbaxa, T = Termicin

Discussion

The cultural, morphological, and biochemical characteristics of isolates D1, D2, and D3 were consistent with Pseudomonas aeruginosa. All isolates produced blue-green to bluish pigment on nutrient agar, were motile Gram-negative rods, and showed positive reactions for catalase, oxidase, cetrimide, and citrate, while indole, methyl red, and Voges-Proskauer tests were negative. These traits aligned with the standard phenotypic description of P. aeruginosa outlined by Cheesbrough (2010), Egberi *et al*. (2026a); Mbanefo *et al*. (2026a) for clinical microbiology laboratories. Although D2 appeared colourless on cetrimide agar, the lack of significant biochemical variation among isolates confirmed phenotypic uniformity. Molecular identification using 16S rRNA sequencing further validated the isolates as P. aeruginosa strains PA03, PA065, and PA076, with 100% identity to reference genomes (Nwadiogbu *et al*., 2026c and Anekwe *et al*., 2026d). This congruence between conventional and molecular methods supports the reliability of 16S rRNA sequencing for definitive identification, as emphasized by Iheukwumere *et al.* (2024)a in studies of environmental and clinical *Pseudomonas* spp.

Antibiotic susceptibility testing revealed high levels of resistance among the *P. aeruginosa* isolates, with 43.59% of 78 tested strains resistant to conventional antibiotics including amoxicillin, penicillin, cephalexin, and sulfamethoxazole-trimethoprim. Multi-antibiotic resistance predominated, accounting for 82.35% of resistant isolates, with PA076 showing 87.50% MDR prevalence. These patterns reflect the intrinsic and acquired resistance mechanisms widely reported in P. aeruginosa, particularly efflux pump overexpression and β-lactamase production (Pang *et al.*, 2019; Anekwe *et al*. 2026c and Madubueze *et al*. 2026d). The observed resistance to β-lactams and aminoglycosides is consistent with findings by Madubueze *et al.* (2026c), who documented 40–60% resistance rates to similar agents among clinical P. aeruginosa in Nigeria. The absence of significant differences in resistance distribution across strains indicated that MDR traits were uniformly disseminated within the sampled population, a trend also noted by Nwike *et al*. (2017) in aquatic *P. aeruginosa* isolates.

Termicin exhibited moderate anti-pseudomonal activity alone, but its combination with Zerbaxa produced marked, ratio-dependent synergy, with 3:7 and 2:8 termicin:Zerbaxa mixtures reducing MICs by 8- to 31.25-fold. At these ratios, MICs were lower than Zerbaxa alone for PA03 and PA065, indicating that sub-inhibitory Zerbaxa potentiated termicin activity. This synergistic interaction aligns with the mechanism proposed by Egberi *et al*. (2026b) and Mbanefo *et al*. (2026b), where insect defensins like termicin disrupt bacterial membranes and facilitate intracellular antibiotic uptake. Similar potentiation of β-lactams by antimicrobial peptides against P. aeruginosa was reported by Madubueze *et al*. (2026b),Nwadiogbu *et al*. (2026d), Egberi *et al*. (2026c) and Nwadiogbu *et al*. (2026e) who observed enhanced ceftazidime activity when combined with cecropin derivatives. The superior efficacy of Zerbaxa-rich combinations suggests that termicin may improve ceftolozane access to penicillin-binding proteins by compromising outer membrane integrity, supporting the utility of peptide–β-lactam dual therapy for MDR *P. aeruginosa* infections.

Conclusion

This study demonstrated significant synergy between termite gut-derived termicin and Zerbaxa against multidrug-resistant *Pseudomonas aeruginosa*, with 3:7 and 2:8 ratios reducing MICs up to 31.25-fold. Sub-inhibitory Zerbaxa potentiated termicin activity, yielding greater potency than either agent alone. These findings provide first evidence of termicin–Zerbaxa synergy and support peptide–β-lactam dual-agent therapy as a promising strategy to overcome *P. aeruginosa* resistance and preserve Zerbaxa efficacy.

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