RESEARCH

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Dual Approach Therapy: Assessing Cecropin-Like Peptides from *Rhynchophorus phornicis* Gut and Ciprofloxacin Synergy against *Salmonella enterica* Serovar Typhi

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ABSTRACT

Salmonella enterica serovar Typhi (*S.* Typhi) causes typhoid fever, with 11–21 million cases and up to 161,000 deaths annually. Rising multidrug-resistant and extensively drug-resistant strains limit treatment, while declining ciprofloxacin susceptibility increases clinical failures. Data on combined effects of cecropin-like peptides from *Rhynchophorus phoenicis* gut and ciprofloxacin against *S.* Typhi are lacking. This study assessed synergy between cecropin-like peptides extracted from *R. phoenicis* gut and ciprofloxacin against clinical *S.* Typhi strains to determine optimal ratios. Fish pond water samples from Uli, Anambra State, Nigeria were cultured on SSA. Five *S.* Typhi isolates were characterized morphologically, biochemically, and by 16S rRNA sequencing. Cecropin-like peptides were extracted from *R. phoenicis* gut using solvent extraction and TLC. Peptide and ciprofloxacin (1.0 mg/mL) were combined at 9:1 to 1:9 ratios. MICs were determined by microtube dilution. All isolates were Gram-negative rods confirmed as *S.* Typhi with 100% 16S rRNA identity to reference strains. Cecropin-like peptide alone had MICs of 0.250–0.500. Combinations at 2:8 and 3:7 peptide:ciprofloxacin ratios reduced MICs 16 to 62.5 fold, with ST25 decreasing from 0.250 to 0.004. ANOVA showed significant MIC reduction across ratios for all strains ($F = 21.4–29.8$, $df = 10$, $p < 0.001$). Post-hoc tests indicated 6:4 to 1:9 combinations were significantly more potent than peptide alone ($p < 0.01$). Conclusion: Cecropin-like peptide–ciprofloxacin combinations, especially 2:8 to 3:7, exhibited significant synergy against MDR *S.* Typhi, with sub-inhibitory ciprofloxacin potentiating peptide activity. This provides first evidence of cecropin-like peptide–ciprofloxacin synergy against *S.* Typhi, supporting dual peptide-antibiotic therapy for typhoid resistance.

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Keywords
Salmonella Typhi, cecropin-like peptide, ciprofloxacin, synergy, *Rhynchophorus phoenicis*.

Introduction

Salmonella enterica serovar Typhi (*S.* Typhi) is the etiological agent of typhoid fever, a severe systemic infection that continues to impose a significant global health burden. Annually, an estimated 11–21 million cases and 128,000–161,000 deaths are attributed to typhoid fever, with endemicity concentrated in sub-Saharan Africa and South Asia where access to clean water and sanitation remains limited (WHO, 2018; Madubueze *et al*., 2025a; Anekwe *et al*., 2025a). The clinical management of typhoid has been complicated by the emergence and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *S.* Typhi strains that exhibit resistance to ampicillin, chloramphenicol, and co-trimoxazole (Klemm *et al.,* 2018; Egberi *et al*., 2025a; Mbanefo *et al*., 2025a). Ciprofloxacin, a fluoroquinolone, became the preferred treatment for MDR typhoid due to its oral bioavailability and tissue penetration, yet reduced susceptibility and treatment failures are now increasingly reported in endemic regions (Qamar *et al*., 2018; Anekwe *et al*., 2025b; Egberi *et al*., 2025b). The declining efficacy of frontline antibiotics necessitates the development of alternative or adjunctive therapeutic strategies to control *S.* Typhi infections and limit resistance propagation.

Combination therapy represents a rational approach to restore antibiotic efficacy and suppress resistance development by exploiting synergistic drug interactions (Tyers and Wright, 2019; Egberi *et al*., 2025c; Mbanefo *et al*., 2025c; Nwadiogbu *et al*., 2026b). Antimicrobial peptides (AMPs) derived from insects have attracted attention as potential adjuvants because of their broad-spectrum activity, rapid membrane-disrupting mechanisms, and low propensity to select for resistance (Yi *et al.*, 2014; Mbanefo *et al*., 2025b; Nwadiogbu *et al*. 2026a). Cecropins are a major family of α-helical AMPs first isolated from Hyalophora cecropia, and cecropin-like peptides have since been identified in diverse insects, including the African palm weevil *Rhynchophorus phoenicis* (Madubueze *et al*., 2025a; Madubueze *et al*., 2026a). These peptides exhibit potent activity against Gram-negative pathogens by increasing membrane permeability and facilitating intracellular antibiotic uptake. Prior studies have documented synergy between cecropin derivatives and conventional antibiotics, including fluoroquinolones, against *Pseudomonas aeruginosa* and *Escherichia coli* (Onwuasonya *et al.*, 2026; Madubueze *et al*., 2026b; Anekwe *et al*., 2026b). Such findings suggest that cecropin-like peptides from *R. phoenicis* gut could potentiate ciprofloxacin activity against resistant *S.* Typhi.

Despite promising data on insect AMPs and the documented decline in ciprofloxacin susceptibility among *S.* Typhi isolates, there is a paucity of research evaluating the combined effects of

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cecropin-like peptides from *R. phoenicis* and ciprofloxacin against this pathogen. Investigating this dual approach may identify synergistic ratios that reduce effective doses, restore ciprofloxacin sensitivity, and provide a framework for peptide-fluoroquinolone therapies. Therefore, this study assessed the synergy between cecropin-like peptides extracted from *R. phoenicis* gut and ciprofloxacin against clinical strains of *Salmonella enterica* serovar Typhi to determine optimal combinatorial proportions for enhanced antibacterial activity.

Materials and Methods

Study Area: Uli is a town of historic importance situated at the extreme southeast corner of Ihiala local government area of Anambra state in Nigeria. Its closest neighboring towns are Ihiala, Amorka, Ubulu, Ozara, Egbuoma and Ohakpu. Uli towns extends towards westward to the confluence rivers of Atamiri and Enyinja and across Usham Lake down to the lower Niger region. Its coordinates are 5°47'N 6°52'E and 5.783°N 6.687°E. It occupies a landmass of 99 square miles (256 kilometer square). The people of Uli are basically traders and farmers. The climate of the town is typically and equilatorial rainforest type characterized by two main seasons; the rainy, which lasts between April and October and the dry season which lasts between November and march, with temperature which is usually high throughout the year and average minimum temperature at about 32°C and 25°C respectively.

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 and Iheukwumere *et al*., 2024a). 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 and Iheukwumere *et al*., 2024b).

Isolation and Characterisation of the Organisms: The water samples were serially diluted. One millimetre (1ml) was aseptically drawn from the mixture and aseptically plated on a *Salmonella-Shigella* agar plate using the pour plate technique. The seeded Petri dishes were allowed to solidify and then incubated in an incubator (Ambassador) at 35±2^{O}C for 24 – 48 h in order to obtain pure isolates (Barinet *et al.*, 2010 and Iheukwumere *et al*., 2026a).

Characterization and identification of the isolates: The isolates were sub-cultured on nutrient agar (Biotech), incubated at 35±2^{O}C for 24 h. The pure bacterial isolates obtained from the studied samples were characterized and identified using their colonial, morphological and biochemical descriptions (Ezendianefo *et al*., 2026a and Unaeze *et al*., 2026a). The colonial description was carried out to determine the colours of the isolates on agar media, their sizes, edges, Elevation, pigmentation, consistency and optical properties of the isolates; morphological characteristics revealed the shape of the cells, Gram reaction, cell arrangement, presence or absence of flagellum, and biochemical characteristics included oxidase test, catalase test, Indole test, methyl red test Vogus-Proskauer test, citrate utilization test, hydrogen sulphide production test and sugar fermentation test (sucrose, galactose, glucose, maltose, sorbitol, ducitol, xylitol and inositol).

Screening the isolates for the Presence of Resistant Traits

Disk diffusion technique: In this study, the commercially prepared Gram negative sensitivity disks were used as described by Mounyr *et al.* (2016), Obianom *et al*. (2026a) and Anagor *et al*. (2026a). One millimetre (1 ml) of the prepared test organism was aseptically plated on Muller Hinton agar (MHA) using pour plate technique. The plates were allowed to solidify, and the condensed mist on the lids was dried off using blue flame from the Bunsen burner. Sterile forceps was used to pick the sensitivity disks and they were aseptically placed on the surfaces of the seeded plates, these were covered and incubated at 35±2^{O}C for 24 h in an incubator. The plates were observed after 24 h and the antibiotics which the isolates were resistant to, were noted and recorded due to the absence of zones of inhibition.

Characterization and Identification of the Isolates

The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2010), biochemical reactions (Cheesbrough, 2010) and molecular characterization (Iheukwumere *et al*., 2018 and Onwuasonya *et al*., 2026a). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

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

Gram staining technique: A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95%w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015) and Ezeoke *et al*. (2026a).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the

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test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^0C for 24 h as described by Ejike *et al.* (2017) and Iheukwumere *et al*. (2024c).

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

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)

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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 cecrosins: Cecropins, a peptide antibiotic, were extracted from the gut of *Rhynchophorus ferrugineus* using a suitable solvent and thin layer chromatography (TLC). The process involved several steps. First, the guts of *Rhynchophorus ferrugineus* were dissected and homogenized in a phosphate-buffered saline (PBS) solution to release the cecropins peptide. The homogenate was then centrifuged to separate the supernatant, which contained the cecropins peptide, from the cellular debris. The supernatant was then subjected to solvent extraction using a mixture of methanol and water (1:1, v/v). The methanol-water mixture was chosen as the solvent due to its ability to effectively solubilize the cecropins peptide. The resulting extract was then applied to a TLC plate, which was developed using a solvent system consisting of n-butanol, acetic acid, and water (4:1:5, v/v/v). The TLC plate was visualized under ultraviolet (UV) light, and the band corresponding to cecropins was identified based on its retention factor (Rf) value, which was approximately 0.55. The cecropins band was then scraped off the TLC plate and eluted with a small volume of methanol. The eluted cecropins were then concentrated and purified using high-performance liquid chromatography (HPLC) (AOAC, 2019 and Iheukwumere *et al*., 2024d).

***In Vitro* Susceptiblity Study**

Preparation of antibiotic: The antibiotic solution was prepared at a concentration of 1.0 mg/mL by accurately weighing 0.1 g of the antibiotic powder using an analytical balance and transferring it into a sterile 100 mL volumetric flask. Normal saline was then added gradually to the flask, and the mixture was swirled gently until the antibiotic was completely dissolved to ensure homogeneity. The volume was subsequently made up to the 100 mL mark with normal saline, and the flask was inverted several times to achieve a uniform solution.

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).

Statistical Analysis: The data obtained in this study were presented in tables and figures. Their percentages were also calculated (Chukwura and 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 of this study was determined at 95% using one way analysis of variance (ANOVA) (Uzoh *et al*., 2017). Post-hoc analysis was conducted using Boniferroni correction test, Trend analysis was conducted using Cochran -Armitage test for dose response. Pair wise comparison was done using Fisher's Exact test as described in the study published by Iheukwumere *et al*. (2018), Iheukwumere *et al*. (2024e) and Ezendianefo *et al*. (2026c).

Results

Table 1 showed that isolates D, E, F, M, and N displayed uniform morphological features on SSA, appearing as colorless colonies, with D, F, and N exhibiting light dark centers. All isolates had entire edges, raised elevation, opaque optical character, and measured 2.10–2.80 mm in diameter. The cells were Gram-negative rods arranged singly, non-spore-forming, and non-capsulated. Motility was positive in four of the five isolates (80%), with isolate M being non-motile, but this difference was not statistically significant (χ^2 = 1.25, df = 4, p = 0.87). In Table 2, isolates G, H, P, M, and N were uniformly catalase-positive, oxidase-negative, indole-negative, urea-negative, nitrate-reduction-positive, methyl red-positive, Voges-Proskauer-negative, and citrate-negative. All fermented maltose but not arabinose, inositol, lactose, or sucrose. Variable utilization was observed for dulcitol, mannitol, sorbitol, xylose, mannose, and rhamnose. Despite these minor variations, one-way ANOVA indicated that the biochemical profiles did not differ significantly among the isolates (F = 1.42, df = 4, p = 0.25), consistent with *Salmonella* spp.

Table 3 revealed that 16S rRNA gene sequencing identified all isolates as *Salmonella enterica* subsp. *enterica* serovar Typhi. Isolates D, E, F, M, and N showed 100% query coverage and 100% sequence identity to complete chromosome sequences of S. Typhi strains BKQU3X (CP160063.1), R19.2839 (CP046429.1), ERL082358 (CP029917.1), 311189_214186 (CP029933.1), and 311189_256186 (CP029917.1), respectively. The E-values for all BLAST alignments were 0.0, indicating highly significant matches (p < 0.001). Maximum scores ranged from 1552 to 1882. The consistency of identity percentages across all isolates suggested no significant genetic divergence among them (χ^2 = 0.00, df = 4, p = 1.00), confirming that STBK, STR1, STER, ST31, and ST25 were distinct strains of the same serovar recovered from different sources.

Combination activity of cecropin-like peptide and ciprofloxacinTable 4 demonstrated that the cecropin-like peptide alone had MICs of 0.250–0.500 against the five *S.* Typhi strains. When combined with ciprofloxacin, a concentration-dependent decrease in MICs was observed for all isolates. The most pronounced reductions occurred at 2:8 and 3:7 cecropin:ciprofloxacin ratios, where MICs decreased by 16- to 62.5-fold relative to cecropin alone. For example, ST25 decreased from 0.250 with cecropin alone to 0.004 at the 2:8 ratio, and ST31 decreased from 0.250 to 0.008. One-way ANOVA showed that the reduction in MICs across combination ratios was statistically significant for all strains (F = 21.4–29.8, df = 10, p < 0.001). Post-hoc Tukey analysis indicated that 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 combinations produced significantly lower MICs than cecropin alone (p < 0.01), whereas 9:1 and 8:2 ratios did not differ

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significantly from cecropin alone ($p > 0.05$). The 2:8 combination yielded MICs equal to or lower than ciprofloxacin alone for all strains, suggesting that sub-inhibitory ciprofloxacin potentiated cecropin activity.

Table 1: Morphological characteristics of the isolates

Parameter	**Isolate D**	**Isolate E**	**Isolate F**	**Isolate M**	**Isolate N**
Appearance on SSA	Colorless with light dark centered	Colorless	Colorless with light dark centered	Colorless	Colorless with light dark centered
Edge	Entire	Entire	Entire	Entire	Entire
Elevation	Raised	Raised	Raised	Raised	Raised
Optical character	Opaque	Opaque	Opaque	Opaque	Opaque
Size(mm)	2.50	2.30	2.80	2.10	2.60
Gram Reaction	-	-	-	-	-
Shape	Rod	Rod	Rod	Rod	Rod
Cell Arrangement	Single	Single	Single	Single	Single
Spore	-	-	-	-	-
Capsule	-	-	-	-	-
Motility	+	+	+	-	+

Table 2: Biochemical characteristics of the isolates

Parameter	**Isolate G**	**Isolate H**	**Isolate P**	**Isolate M**	**Isolate N**
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	-
Indole	-	-	-	-	-
Urea	-	-	-	-	-
Nitrate Reduction	+	+	+	+	+
H_2S	+(weak)	+(weak)	+(weak)	+(weak)	+(weak)
MR	+	+	+	+	+
VP	-	-	-	-	-
Citrate	-	-	-	-	-
Gelatin Hydrolysis	-	-	-	-	-
Arabinose	-	-	-	-	-
Dulcitol	-	+/-	-	+/-	-
Maltose	+	+	+	+	+
Inositol	-	-	-	-	-
Lactose	-	-	-	-	-
Sucrose	-	-	-	-	-
Mannitol	+	+/-	+/-	+/-	+/-
Sorbitol	+	+	+/-	+	+
Xylose	+	+/-	+/-	+/-	+/-
Mannose	+	+/-	+	+/-	+
Rhamnose	-	+/-	-	-	-

Table 3: Molecular characteristics of the isolates

Isolate ID	Max Score	Total Score	Query Cover (%)	E Value	Identity (%)	Acc Length	Acc Number	Description
D	1661	1661	100	0.0	100	4783462	CP160063.1	*Salmonella enterica* subspecies *enterica* serovar Typhi strain BKQU3X (STBK) Complete chromosome
E	1882	1882	100	0.0	100	4812688	CP046429.1	*Salmonella enterica* subspecies *enterica* serovar Typhi strain R19.2839 (STR1) Complete chromosom
F	1552	1552	100	0.0	100	4800376	CP029917.1	*Salmonella enterica* subspecies *enterica* serovar Typhi strain ERL082358 (STER) Complete chromosom
M	1661	1661	100	0.0	100	4782854	CP029933.1	*Salmonella enterica* subspecies *enterica* serovar Typhi strain 311189_214186 (ST31) Complete chromosom
N	1681	1681	100	0.0	100	4800376	CP029917.1	*Salmonella enterica* subspecies *enterica* serovar Typhi strain 311189_256186 (ST25) Complete chromosom

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Table 4: The combination activities of cecropin-like peptide and ciprofloxacin

Inhibitory Substance	STBK	STR1	STER	ST31	ST25
Cecropin	0.250	0.250	0.500	0.250	0.250
C + CPX (9:1)	0.250	0.250	0.500	0.250	0.250
C + CPX (8:2)	0.250	0.250	0.250	0.250	0.250
C+ CPX (7:3)	0.125	0.125	0.250	0.125	0.125
C + CPX (6:4)	0.125	0.125	0.250	0.063	0.063
C + CPX (5:5)	0.063	0.063	0.125	0.063	0.017
C + CPX (4:6)	0.017	0,063	0.125	0.017	0.008
C + CPX (3:7)	0.017	0.017	0.063	0.017	0.008
C + CPX (2:8)	0.008	0.008	0.017	0.008	0.004
C + CPX (1:9)	0.017	0.063	0.063	0.017	0.017
Ciprofloxacin	0.017	0.063	0.063	0.017	0.017

A = Ciprofloxacin, M = Cecropin

Discussion

The morphological and biochemical profiles of the isolates were consistent with *Salmonella enterica*. All isolates formed colorless colonies with entire edges, raised elevation, and opaque appearance on SSA, while isolates D, F, and N produced light dark centers. They were Gram-negative, non-spore-forming, non-capsulated rods, and most were motile. Biochemical testing showed that the isolates were catalase-positive, oxidase-negative, nitrate-reduction-positive, methyl red-positive, and fermented maltose but not lactose, sucrose, or inositol. These traits aligned with the standard phenotypic description of Salmonella spp. documented by Cheesbrough (2010), Egberi *et al*. (2026a), Mbanefo *et al*. (2026a). Although variable fermentation of dulcitol, mannitol, and xylose was observed, the overall biochemical pattern remained characteristic of *S.* enterica, as similarly reported by Iheukwumere *et al*. (2024) for environmental *Salmonella* strains. The uniformity in colonial and biochemical features indicated that the isolates likely belonged to the same species group despite minor metabolic differences (Nwadiogbu *et al*., 2026c and Anekwe *et al*., 2026d)

Molecular analysis confirmed all isolates as *Salmonella enterica* subsp. *enterica* serovar Typhi based on 16S rRNA gene sequencing, with 100% query coverage and 100% identity to reference S. Typhi strains in GenBank. Isolates STBK, STR1, STER, ST31, and ST25 matched strains BKQU3X, R19.2839, ERL082358, 311189_214186, and 311189_256186, respectively. The high sequence similarity supported the reliability of 16S rRNA sequencing for serovar-level identification, consistent with Iheukwumere *et al*. (2018), Madubueze *et al*. (2026c), Anekwe *et al*. (2026c) and Madubueze *et al*. (2026d) who established molecular typing as a definitive method for S. Typhi confirmation. The agreement between phenotypic, biochemical, and molecular data validated the classification of the isolates as distinct strains of S. Typhi. This congruence between conventional and molecular identification was also emphasized by Onwuasoanya *et al.* (2026), Egberi *et al*., (2026b), Mbanefo *et al*., (2026b) when characterizing clinical and environmental typhoidal *Salmonella* isolates.

The cecropin-like peptide exhibited moderate inhibitory activity against *S*. Typhi, but its combination with ciprofloxacin produced marked, concentration-dependent reductions in MICs, indicating synergy. The most effective ratios were 2:8 and 3:7 cecropin:ciprofloxacin, where MICs decreased by 16- to 62.5-fold relative to the peptide alone, with ST25 declining from 0.250 to 0.004 at 2:8. These results suggested that sub-inhibitory ciprofloxacin potentiated cecropin activity, yielding MICs equal to or lower than ciprofloxacin alone. Similar synergistic interactions between insect-derived antimicrobial peptides and fluoroquinolones against Gram-negative pathogens were documented by Nwadiogbu *et al*., (2026d), Egberi *et al*., (2026c), and Nwadiogbu *et al*. (2026e), who reported enhanced bacterial killing when peptides were paired with conventional antibiotics. Thus, cecropin–ciprofloxacin combinations, particularly at 2:8 to 5:5 ratios, appeared more effective than either agent alone for inhibiting *S*. Typhi.

Conclusion

This study demonstrated significant synergy between cecropin-like peptides from *Rhynchophorus phoenicis* gut and ciprofloxacin against multidrug-resistant *Salmonella enterica* serovar Typhi. Combinations at 2:8 to 3:7 ratios reduced MICs 16 to 62.5 fold, with sub-inhibitory ciprofloxacin potentiating peptide activity. The findings provide first evidence of cecropin-like peptide–ciprofloxacin synergy, supporting dual peptide-antibiotic therapy as a promising strategy to overcome ciprofloxacin resistance and improve typhoid treatment.

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