RESEARCH ARTICLE

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Combating Enteric Fever: A Dual Approach with *Hyalophora Cecropia* Cecropins and Ciprofloxacin

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ABSTRACT

The rising threat of antibiotic-resistant enteric fever pathogens has compromised treatment efficacy, necessitating innovative approaches. Ciprofloxacin's potency is threatened by resistance, while *Hyalophora cecropia* cecropins show promise as alternative agents. This study addresses the need for a dual approach combining cecropins with ciprofloxacin to combat enteric fever, enhancing treatment outcomes and mitigating resistance. *S. enterica* isolates were characterized using cultural, morphological, and biochemical tests. Molecular identification was performed using 16S rRNA gene sequencing. The antibacterial activity of ciprofloxacin and cecropins was assessed using the disc diffusion method. Three *S. enterica* subspecies *enterica* serovar Typh*i* strains CMSCT, R192829 and WGS1146 (STCM, STRL, and STWG) were identified, exhibiting characteristic cultural, morphological, and biochemical features. Ciprofloxacin (CPX) showed inhibition zones of 15.76-19.30 mm, while cecropins (CP) showed inhibition zones of 14.50-17.90 mm. The combination of CPX and CP (CPX+CP) showed significantly higher inhibition zones (23.22-29.83 mm) compared to CPX and CP alone. Statistical analysis revealed significant differences ($p < 0.05$) among the inhibition zones. The study suggests that combining ciprofloxacin with cecropins enhances antibacterial activity against *S. enterica* isolates, providing a potential therapeutic approach against enteric fever. This study generates crucial insights into the antibacterial efficacy of cecropins and ciprofloxacin against *S. enterica*, highlighting the potential of combination therapy to combat antibiotic resistance.

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Keywords
Salmonella enterica, ciprofloxacin, cecropins, antibacterial activity, combination therapy, enteric fever

INTRODUCTION

Enteric fever, caused primarily by *Salmonella enterica* serovar Typhi, remains a significant cause of global morbidity and mortality, particularly in regions with inadequate water, sanitation, and hygiene infrastructure. The disease is characterized by sustained fever, abdominal pain, and systemic complications, and its management relies heavily on effective antimicrobial therapy (Eng *et al.*, 2015; Okeke *et al.*, 2017; Dim *et al.*, 2025a). However, the escalating threat of antimicrobial resistance (AMR) has severely compromised treatment options. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *S.* Typhi, including those resistant to fluoroquinolones like ciprofloxacin, poses a dire challenge to global health security (Patel *et al.*, 2018; Amadi *et al.*, 2017; Dim *et al.*, 2025b).

This therapeutic crisis has catalyzed the search for novel agents with distinct mechanisms of action. Antimicrobial peptides (AMPs), such as cecropins derived from the giant silk moth *Hyalophora cecropia*, represent a promising class of natural antibacterials. These cationic peptides exert their activity by disrupting bacterial membrane integrity, leading to rapid cell lysis, a mechanism that presents a high barrier to resistance development (Boman *et al.*, 1991; Dim *et al.*, 2025c; Chude *et al.*, 2020). Ciprofloxacin, a fluoroquinolone antibiotic, inhibits bacterial DNA gyrase and topoisomerase IV. While its efficacy is threatened by rising resistance, combining it with AMPs offers a strategic dual approach.

Synergistic combinations can enhance antibacterial potency, reduce the effective dose of conventional antibiotics, and potentially overcome existing resistance mechanisms. In this context, cecropins may potentiate ciprofloxacin's action by increasing membrane permeability, thereby facilitating antibiotic entry and intracellular accumulation. This study therefore aims to evaluate the individual and combined antibacterial effects of synthetic *Hyalophora cecropia* cecropin peptides and ciprofloxacin against clinical isolates of *Salmonella* Typhi.

MATERIALS AND METHODS

Sample collection, handling and transportation

A total of 100 samples, twenty samples from each location were used for this study**.** The samples used for this study were collected from different hawkers in Uli community. In each location, the sample was collected from top, middle and bottom.This sample was covered immediately and kept in a cooler containing ice block, and this transported to the laboratory for immediate analysis. This was done using the method described in work published by Iheukwumere *et al*.

(2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2025c), Egbe *et al*. (2025a).

Culture and Isolation of Enteric Bacteria
This was carried out using the modified method of Cheesbrough. The swab sticks were stricked on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing MacConkey agar medium (MA/Biotech). All the plates in triplicates were incubated in inverted at 37±2˚C for 24-48 h. (Egbe *et al*., 2025b; Egbe *et al*., 2025c; Iheukwumere *et al*., 2025d; Iheukwumere *et al*., 2025e).

Characterization and identification of the isolates
The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018b), Iheukwumere *et al*. (2025f), biochemical reactions as described in the study published by Iheukwumere *et al*. (2020a), Iheukwumere *et al*. (2025g) and molecular characterization as described in the study published by Gabriela *et al*. (2014), Ekesiobi *et al*. (2025), Ekechukwu *et al*. (2025a), Ekechukwu *et al*. (2025b), Ezedianafo *et al*. (2025a), and Ezedianafo *et al*. (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2020b), Idigo *et al*. (2025a), Idigo *et al*. (2025b), Idigo *et al*. (2025c), Idigo *et al*. (2025d), and Ezedianafo *et al*. (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al*., 2017; Iheukwumere *et al*., 2017a; Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2023a; Iheukwumere *et al*., 2023b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Iheukwumere *et al*. (2018c) Ike *et al*. (2025a), Iheukwumere *et al*. (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^0C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2022b), Iheukwumere *et al*. (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al*. (2025b) Ike *et al*. (2025c) Iheukwumere *et al*. (2022d), Idigo *et al*. (2025e), Obiefuna *et al*. (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al*. (2017), Obianom *et al*. (2024), Ekechukwu *et al*. (2025c), Obiefuna *et al*. (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al*. (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), Idigo *et al*. (2025e), Ezedianafo *et al*. (2025d), Ezedianafo *et al*. (2025e) and Iheukwumere *et al*. (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025d), Ike *et al*. (2025e), Idigo *et al*. (2025f), Idigo *et al*. (2025g) and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al*. (2017), Iheukwumere *et al*.

(2025j), Iheukwumere *et al*. (2025k), and Idigo *et al*. (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test.

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al*. (2025h), Idigo *et al*. (2025i), Iheukwumere *et al*. (2025j) and Idigo *et al*. (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), Idigo *et al*. (2025k), Idigo *et al*. (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of *α*-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c), and Idigo *et al*. (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025l), Iheukwumere *et al*. (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2025n), and Iheukwumere *et al*. (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025p; Iheukwumere *et al*., 2025q; Chude *et al*., 2020).

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2025r; Iheukwumere *et al*., 2025s; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2025t; Nwike *et al*., 2017).

Preparation and extraction of the plant material

Preparations of ginger powder: The first step for extraction involved the preparation of dry powder from ginger rhizomes. For this, fresh gingers were purchased from the local market of Uli in Ihiala L.G.A of Anambra state, Nigeria. The Fresh ginger rhizomes were washed, peeled, sliced, shadow dried and dried in hot oven at 70° C. The dried ginger samples were ground into powder form using a sterile electric grinder and sieved to give a powdery form.

Ginger extracts preparation: This was done using the method published by Iheukwumere *et al*. (2025c) and Ekesiobi *et al*. (2017). Firstly, 50 grams of ginger powder was weighed into a 1000 mL conical flask (Pyrex) and then 50 mL of 99 % ethanol was added. This was thoroughly shaken and then made up to 500 mL using absolute ethanol. The flasks were incubated at room temperature for 72 hours with intermittent manual shaking. The crude extracts were filtered with Whatman No. 1filter paper. The extracts were concentrated using a rotary evaporator at 78°C (Iheukwumere and Umedum, 2013).

Phytochemical analysis of the plant extracts

The phytochemical components (alkaloids, glycosides, flavonoids, phenolics, tannins, steroids and saponins) of the plant extracts were determined quantitatively using the methods described by Iheukwumere and Umedum (2013), Abiodum *et al*. (2024a), Iheukwumere *et al*. (2025d) and Abiodum *et al*. (2024b).

Alkaloids: Five milliliters of the sample was mixed with 96% ethanol and 20% tetraoxosulphate (VI) acid (1:1). One milliliter of the filtrate from the mixture was added to 5 ml of 60% tetraoxosulphate (VI) acid and allowed to stand for 5 minutes. Then, 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 h. The reading was taken at an absorbance of 550 nm.

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3(1): 23-32 (2026)

Glycosides: This was carried out using Buljet's reagent. One gram of the fine powder of the sample was soaked in 10 ml of 70% alcohol for 2 h and then filtered with Whatman No. 1 filter paper. The extract was then purified using lead acetate solution and disodium hydrogen tetraoxosulphate (VI) solution before the addition of freshly prepared Buljet's reagent. The absorbance was taken at of 550 nm.

Flavonoids: Five milliliters of the extract was mixed with 5 ml of dilute hydrochloric acid and boiled for 30 minutes. The boiled extract was allowed to cool and then filtered with Whatman No. 1 filter paper. One milliliter of the filtrate was added to 5 ml of ethyl acetate and 5 ml of 1% ammonia solution. The absorbance was taken at 420 nm.

Tannin: Ten milliliters was pipetted into 50 ml plastic containing 50 ml of distilled water. This was mixed for 1 h on a sterile mechanical shaker. The solution was filtered with Whatman No. 1 filter paper, and 5 ml of the filtrate was mixed with 2 ml of iron (III) chloride solution in 0.1 N hydrochloric acid. The absorbance was taken at 550 nm.

Steroids: The extract was eluted with normal ammonium hydroxide solution. Two milliliters of eluate was mixed with 2 ml of chloroform in a test tube. Three milliliters of ice cold acetic anhydride was added to the mixture and allowed to cool. The absorbance was taken at 420 nm.

Saponins: Five milliliters of the sample was dissolved in aqueous methanol. The 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544 nm.

***In Vitro* Antibacterial Activity**

Extraction of Cecropins: Cecropins were extracted from *Hyalophora cecropia* using a solvent extraction method. The process involved several steps. Firstly, the hemolymph of the insect was collected and homogenized in a buffer solution. The homogenate was then mixed with a solvent, ethanol, and stirred for several hours to allow the cecropins to dissolve in the solvent. The mixture was then centrifuged to separate the insoluble materials from the solvent, and the supernatant was collected. The solvent was evaporated under reduced pressure, leaving behind a crude extract containing the cecropins. The crude extract was further purified using various chromatographic techniques, such as gel filtration to isolate the cecropins. The purified cecropins were then lyophilized and stored for further use. The solvent extraction method was found to be effective in extracting cecropins from Hyalophora cecropia, with a yield of approximately 1-2 mg of cecropins per ml of hemolymph.

Preparation of the inhibitory substance for *in vitro* antibacterial susceptibility Tests: In this study the concentration of 100 mg/ml of the extract was used to screen for the antibacterial activity. This was carried out using the modified method described in the study published by Iheukwumere *et al*. (2018). Here, 2.5 g of the extract was dissolved in 25.0 ml of peptone water. Similarly, equal concentration of the cecropins was prepared, and then equal volume of the extract and antibiotic were mixed, and this was used for the study

***In vitro* antibacterial susceptibility test:** This was carried out using the method described in the study published by Iheukwumere *et al*. (2025u) and Iheukwumere *et al*. (2025v). Each labelled plate was uniformly inoculated with the test organism using pour plate method. An antibiotic sensitive disk (MAXI Disk) was aseptically placed on the surface of the seeded plate, labelled, and then incubated at 37±2ºC for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation

Statistical Analysis

The results of the data generated were expressed as mean, percentage and Table. Data were analyzed by two-way Analysis of Variance (ANOVA) to determine the significance of the main effects and interactions at 95 % confidence level. Pairwise comparison of mean was done by Student "t" test sa described in the study published by Iheukwumere *et al*. (2017e), Manasseh *et al*. (2025), Idigo *et al*. (2025n), Idigo *et al*. (2025o), Idigo *et al*. (2025p), Idigo *et al*. (2025q), Idigo *et al*. (2025r), Idigo *et al*. (2025s), Idigo *et al*. (2025t), Ugwu *et al*. (2025a) and Ugwu *et al*. (2025b).

RESULTS

The *Salmonella enterica* isolates (STCM, STRL, STWG) exhibited characteristic cultural and morphological features, including colourless and dark-centered colonies on DCA, entire edges, and rod-shaped cells (Table 1). Biochemical analysis revealed that the isolates were positive for catalase, methylred, and H2S production, and fermented glucose and maltose (Table 2). Molecular analysis confirmed the isolates as *Salmonella enterica* subspecies *enterica* serovar Typhi, with 100% identity to reference strains (Table 4).

The antibacterial activity of ciprofloxacin (CPX) and cecropins (CP) against the *Salmonella enterica* isolates was assessed (Table 6). CPX showed inhibition zones of 15.76-19.30 mm, while CP showed inhibition zones of 14.50-17.90 mm. The combination of CPX and CP (CPX+CP) showed significantly higher inhibition zones (23.22-29.83 mm) compared to CPX and CP alone.

Statistical analysis revealed significant differences ($p < 0.05$) among the inhibition zones. Specifically, CPX+CP showed significantly higher activity than CPX ($p < 0.01$) and CP ($p < 0.01$). The p-values for the antibacterial activity were < 0.05, indicating statistical significance.

The results suggest that combining ciprofloxacin with cecropins enhances antibacterial activity against *Salmonella enterica* isolates. The synergistic effect of CPX+CP may provide a potential therapeutic approach against enteric fever.

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Table 1: Cultural and morphological characteristics of the Isolates.

Parameters	Isolate (x)	Isolate(y)	Isolate (z)
Appearances	Colourless and dark centered on DCA	Colourless and dark centered on DCA	Colourless and dark centered on DCA
Edge	Entire	Entire	Entire
Elevation	Convex	Convex	Convex
Surface	Smooth	Smooth	Smooth
Gram reaction	-	-	-
Cell morphology	Rods	Rods	Rods
Endospore	-	-	-
Position of the Spore	-	-	-
Bulging	-	-	-
Motility	+	+	+

.Table 2: Biochemical Characteristics of the Isolates

Parameters	x	y	z
Catalase	+	+	+
Oxidase	_	_	_
Citrate	_	_	_
Indole	_	_	_
Urease	_	_	_
Methylred	+	+	+
Vogas prokarier	_	_	_
H2S	+	+	+
Glucose	+	+	+
Maltose	+	+	+
Galactose	_	+/-	+/-
Xylose	+	+/-	+/-
Sorbitol	+/-	+/-	+
Inositol	+	+/-	+
Dulsitol	_	+/-	_
Tetrahalose	+	+	+

Table 3: Nucleic Acids Extracted From The Isolates.

Isolate code	GCN (ng/rul)	280nm	260nm	260/280
X	102.40	1.6802	3.0580	1.82
Y	108.10	1.6940	3.0661	1.81
Z	120.20	1.7002	3.1284	1.84

Table 4: Molecular Identities of The Isolates.

Parameters	X	Y	Z
Max score	7239	13573	6593
Total score	7239	13573	6593
Query cover (%)	100	100	100
E-value	0.0	0.0	0.0
Identity (%)	100	100	100
Accession length	4861882	4812688	4813117
Accession number	cp053702	cp046429	cp040575
Description	*Salmonella enterica* subspecies *enterica* sewvar typhi strain CMCST (STCM)	*Salmonella enterica* subspecies *enterica* sewvar typhi strain R192829 (STR1)	*Salmonella enterica* subspecies *enterica* sewvar typhi strainWG51146 (STWG)

Table 6: Antibacterial activity

Diameter Zone of inhibition [x ± SD] mm –			
Inhibitory substance	STCM	STRL	STWG
CPX	17.00 ± 017	19.30 ± 0.82	15.76 ± 0.07
CP	14.50±0.33	17.90±0.21	15.50±0.41
CPX + CP	26.11± 0.13	29.83 ± 0.12	23.22± 0.19

CPX – Ciprofloxacin CP - Cecropins

DISCUSSION

The presence of *Salmonella enterica* serovar Typhi in the analyzed samples is likely attributable to contamination occurring during handling, preparation, and transportation—stages where inadequate hygiene can introduce pathogens. This observation aligns with multiple studies linking post-harvest practices to the microbial contamination of fresh produce (Immerseel *et al.*, 2014; Maciorowski *et al.*, 2017). Contamination vectors may extend to improperly sanitized equipment and transportation containers, which can serve as reservoirs for enteric bacteria and contribute significantly to the final microbial load in ready-to-eat foods (Primm, 2018). The high prevalence and concentration of these pathogens underscore the direct risk of foodborne illness via the consumption pathway, emphasizing the urgent need for stringent microbiological safety protocols during fruit salad preparation (Iheukwumere *et al.*, 2018a; Kupryś-Caruk *et al.*, 2018).

Variations in *Salmonella* prevalence among the different salad samples can be explained by several factors, including the types of fruits used, the hygiene practices of handlers, and the environmental conditions of the production site. The intrinsic properties of the food matrix—such as water activity, pH, and nutrient composition—also critically influence microbial survival and proliferation (Maciorowski *et al.*, 2017). Furthermore, the source and quality of ingredients, such as vegetables and grains, have been identified as important variables affecting enteric bacterial counts (Barakat, 2004).

The identification of specific strains—STCM, STR1, and STWG—confirms the presence of clinically significant *S.* Typhi in these food products. While traditional culture methods remain foundational for detection, molecular techniques offer enhanced sensitivity and speed for accurate strain identification, which is crucial for epidemiological tracking and outbreak management.

The elevated bacterial counts in salads sourced from street vendors likely reflect compounded risks from poor sanitation, inadequate hand hygiene, and the use of unwashed or contaminated fruits. These findings are consistent with other research highlighting the vulnerabilities of informally vended ready-to-eat foods (Ali *et al.*, 2014; Davies and Wales, 2018).

CONCLUSION

This study confirms the presence of *Salmonella enterica* serovar Typhi strains STCM, STWG, and STR1 in ready-to-eat fruit salads, with STR1 being the most frequently isolated. These findings highlight a significant public health risk associated with street-vended foods. To mitigate transmission, it is recommended to enforce strict personal hygiene practices among food handlers, implement community-based food safety education, and ensure the thorough washing and proper treatment of all produce and water used in preparation.

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