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Synergistic Evaluation of Ciprofloxacin with Plant Essential Oils against Salmonella Spp. Isolated from Animal Origin

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Abstract

Foodborne salmonellosis caused by *Salmonella* spp. represented a major public health concern, further complicated by multidrug-resistant (MDR) strains. This study assessed the distribution, antibiotic resistance, and synergistic potential of plant essential oils (EOs) with ciprofloxacin (CIP) against *Salmonella* isolates from animal-based food samples collected in Faisalabad, Pakistan. Out of 105 samples, 41 (39%) were found positive for *Salmonella*, with the highest distribution in beef (73%) and mutton (67%). Antibiotic susceptibility testing revealed high resistance to co-trimoxazole (78%), ampicillin (76%), and ceftriaxone (76%), while CIP resistance was observed in 49% of isolates. Four EOs clove (CEO),

thyme (TEO), tea tree (TTEO), and rosemary (REO)—were evaluated for antibacterial activity; CEO exhibited the largest inhibition zones (20–24 mm) and the lowest MIC values (250–500 µg/mL).Checkerboard assays showed that CIP–CEO combinations produced the strongest synergistic effect (FICI 0.39–0.47), CIP–TTEO displayed partial synergy (FICI 0.72–0.75), CIP–TEO demonstrated additive effects (FICI 0.88–0.92), and CIP–REO combinations were antagonistic (FICI 1.10–1.19). These results indicated that specific EOs, particularly CEO and TTEO, enhanced CIP efficacy against MDR *Salmonella* spp., suggesting a potential strategy to reduce antibiotic doses and mitigate antimicrobial resistance.

Keywords: Salmonella spp., Ciprofloxacin, Essential oils, Synergistic effect, foodbornemicrobes

Introduction

Foodborne salmonellosis remains one of the most critical global public health concerns, caused predominantly by *Salmonella spp*. infections, transmitted primarily through contaminated animal-based food products such as poultry, beef, mutton, and seafood. According to the World Organization (WHO), non-typhoidal Salmonella infections account for approximately 93.8 million gastroenteritis cases and over 150,000 deaths annually, with 85% of cases linked to food consumption. The increasing incidence of antimicrobial-resistant (AMR) strains has further amplified the challenge of managing Salmonella infections (Geresu, Wayuo et al. 2021).

AMR poses a major global threat, particularly in foodborne pathogens. The misuse and overuse of antibiotics in poultry and livestock production have led to the emergence of multidrug- resistant (MDR) Salmonella strains. Ciprofloxacin (CIP), a broad-spectrum fluoroquinolone, has been one of the most effective agents against Salmonella infections (Şık, Altıntaş et al. 2022). However, increasing resistance to CIP has been reported in multiple regions, necessitating alternative or adjunctive therapeutic approaches (Sallam, Kasem et al. 2024).

EOs extracted from aromatic plants are recognized for their broad-spectrum antibacterial, antifungal, and antioxidant properties. Compounds such as eugenol in clove oil (CEO), thymol and carvacrol in thyme oil (TEO), terpinen-4-ol in tea tree oil (TTEO), and camphor in rosemary oil (REO) disrupt bacterial cell membranes and

inhibit metabolic functions (Bo, Zhan et al. 2023). These bioactive components have shown strong efficacy against resistant bacterial species, making them promising candidates for synergistic studies with antibiotics.

The concept of combining EOs with conventional antibiotics offers potential to restore the efficacy of existing antimicrobials by reducing required dosages and minimizing resistance development. However, limited data are available on the synergistic interactions between ciprofloxacin and various EOs against foodborne *Salmonella spp.* Therefore, this study aims to evaluate the synergistic potential of ciprofloxacin with clove, thyme, tea tree, and rosemary EOs against *Salmonella spp*. isolated from animal-based food sources.

Materials and Methods

Study Design and Sampling

This cross-sectional experimental study was carried out at the Institute of Microbiology, Government College University, Faisalabad. A total of 105 animal-based food samples, including poultry, beef, mutton, fish, and prawns, were aseptically collected from slaughterhouses and retail markets in Faisalabad. Samples were transported in sterile containers to the laboratory under refrigerated conditions for microbiological examination.

Isolation and Identification of *Salmonella spp.*

Each sample was firstly enriched in Selenite F broth at 37°C for 24 h and sub-cultured on Salmonella-Shigella (SS) agar and Xylose Lysine Deoxycholate (XLD) agar (OXOID®, Basingstoke, UK), and incubation was done for 24 h at 37°C Typical colonies were examined microscopically using Gram staining and biochemical characterization.

Antibiotic Susceptibility Testing

Antibiotic susceptibility profiles were determined using the Kirby–Bauer disc diffusion method on Mueller Hinton agar, in accordance with CLSI guidelines. Tested antibiotics included ciprofloxacin (5 µg), azithromycin (15 µg), co-trimoxazole (25 µg), chloramphenicol (30 µg), ampicillin (10 µg), and ceftriaxone (30 µg). Zone diameters were measured to categorize isolates as sensitive or resistant.

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Antibacterial Activity of Essential Oils

The antibacterial efficacy of four essential oils—clove (CEO), thyme (TEO), tea tree (TTEO), and rosemary (REO) were tested using the agar well diffusion method. Each well received 100 µL of EO prepared in dimethyl sulfoxide (DMSO), and plates were incubated at 37°C for 24 hours. Zone diameters were measured to assess inhibitory activity (Tan, Nordin et al. 2022).

Determination of MIC, MBC, and Synergistic Activity

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of EOs and ciprofloxacin were determined using the broth microdilution technique in 96-well plates. Bacterial growth was evaluated using nitro-blue tetrazolium (NBT) dye (Edwin, Osarobo et al. 2023). Synergistic potential between ciprofloxacin and EOs was assessed through the checkerboard assay, and the fractional inhibitory concentration index (FICI) was calculated. FICI
$\leqslant$ 0.5 indicated synergy, 0.5–1.0 additivity, and >1.0 no interaction.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using Microsoft Excel, employing descriptive statistics and one-way ANOVA to evaluate significance at $p < 0.05$.

Results

Distribution of *Salmonella spp*. in Food Samples

Out of 105 food samples tested, 41 (39%) were positive for *Salmonella spp*. The highest prevalence was observed in beef (73%), followed by mutton (67%), chicken (53%), fish (47%), and prawns (33%). No *Salmonella spp*. were isolated from eggs and cloacal swabs as shown in Table 1.

Table 1: *Distribution Of Salmonella Spp.From Different Animal-Based Food Sources*

Category	Sample Type	No. of Samples	Positive Samples	Distribution of *Salmonella spp* (%)
Livestock	Beef	15	11	73%
	Mutton	15	10	67%
Fisheries	Prawn	15	5	33%

	Fish	15	7	47%
Poultry	Chicken	15	8	53%
	Poultry Eggs	15	0	0
	cloacal Swabs	15	0	0
Total	—	105	41	39%

Antibiotic Resistance Profile

High resistance rates were recorded against co-trimoxazole (78%), ampicillin (76%), ceftriaxone (76%), and chloramphenicol (66%). Ciprofloxacin resistance was noted in 49% of isolates, while azithromycin exhibited the lowest resistance (24%). These results demonstrate a multidrug- resistant pattern among Salmonella isolates (Figure 1).

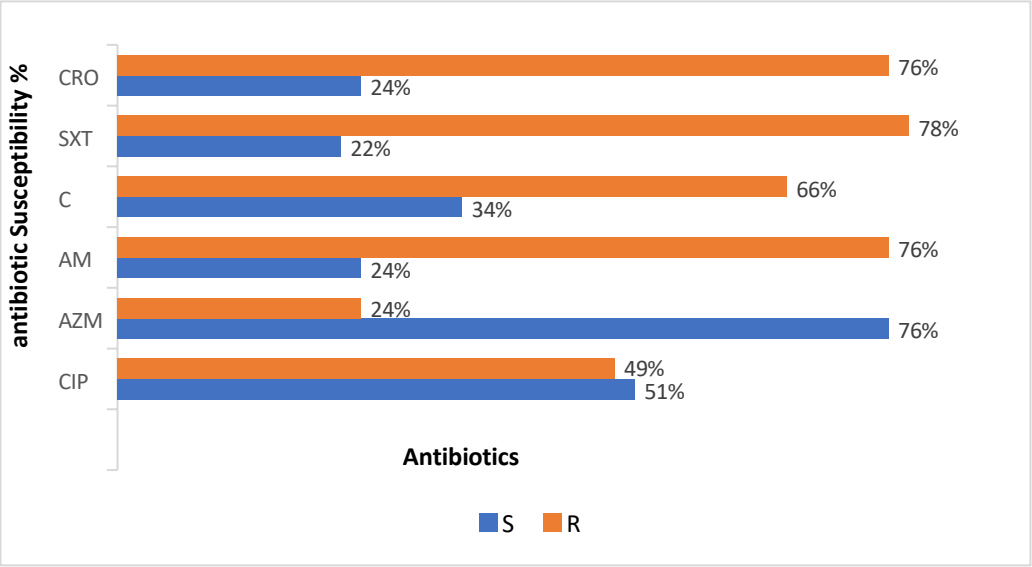

Figure 1: Thebargraph shows antibiotic susceptibility percentage of various antibitiocin Salmonella spp. from various animal based food sources.

Antibacterial Efficacy of Essential Oils

Among the tested EOs, CEO exhibited the highest antibacterial activity with inhibition zones ranging between 20–24 mm, followed by TEO (18–22 mm), TTEO (18–20 mm), and REO (16–19mm). MIC values for all EOs ranged from 25 to 250 µg/mL, and ciprofloxacin showed an MIC of 1 µg/mL against all isolates.

The Checkerboard Assay

Checkerboard assays indicated that ciprofloxacin combined with CEO demonstrated thestrongest synergistic interaction with FICI values ⩽ 0.5, while TEO and TTEO combinations showed additive effects (FICI 0.8–1.0). The combination of REO and ciprofloxacin revealed limited or no synergism (FICI ⩾ 1.0). Overall, the study confirmed that specific essential oils can enhance the antibacterial potency of ciprofloxacin against *Salmonella spp*. isolated from animal origin.

Discussion

AMR due to *Salmonella spp*. from animal origin has emerged as a major global health concern. The widespread and indiscriminate use of antibiotics in both animal husbandry and human medicine has led to the evolution of MDR strains, posing severe challenges in disease management. In this study, a high distribution of antibiotic-resistant *Salmonella* isolates was observed, particularly against co-trimoxazole, ampicillin, and ceftriaxone, findings that correspond with previous reports by Fatima, Sadiq, et al. (2025), who described similar resistance patterns in meat-derived *Salmonella* isolates from Pakistan. These results reaffirm the critical need for alternative antimicrobial strategies that can either replace or complement conventional antibiotics.

EOs including CEO, TEO, TTEO, and REO , demonstrated promising antibacterial activity in this study. CEO showed the largest inhibition zones and the lowest MIC values, aligning with the findings of (Huerta Lorenzo, Galán-Relaño et al. 2024) and (Zahli, El Baaboua et al. 2024), who reported comparable results for *Salmonella* spp. Similarly, moderate antimicrobial activity was recorded for TEO and TTEO, while REO exhibited comparatively lower activity, requiring higher concentrations to inhibit bacterial growth. These variations in antibacterial potency among oils are likely attributed to differences in their phytochemical compositions, primarily the concentration of eugenol, thymol, and terpenoids.

Checkerboard assays confirmed that combining ciprofloxacin (CIP) with CEO produced the strongest synergistic effect (FICI ⩽ 0.5), followed by TTEO and TEO, which exhibited partial synergy. However, CIP-REO combinations displayed antagonistic interactions, indicating that not all EO-antibiotic pairings yield favorable

results. These outcomes are consistent with (Soulaimani 2025) and (Rosato, Napoli et al. 2023), who highlighted that while certain EOs enhance antibiotic efficacy, others may hinder it depending on molecular compatibility and bacterial resistance mechanisms.

Overall, this study supports the potential use of EOs, particularly CEO and TTEO, as effective natural adjuncts to conventional antibiotics. Their synergistic interactions with CIP could allow for reduced antibiotic dosages, minimize side effects, and slow the progression of AMR. Such combination therapies hold promise for improving food safety and public health by offering eco- friendly, sustainable alternatives to synthetic antimicrobials.

Conclusion

This study demonstrated that plant EOs, particularly clove and thyme, possess significant antibacterial activity and exhibit synergistic interactions with ciprofloxacin against *Salmonella spp* from animal based food origin. The combination therapy effectively enhanced antibacterial potency, reduced MIC values, and offered a potential solution to mitigate antibiotic resistance in foodborne pathogens. Future research should explore in vivo applications and molecular mechanisms underlying these synergistic effects to develop natural, sustainable alternatives to conventional antibiotics.

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