

# Evaluation of the Antibacterial Synergism of Chloramphenicol and Co-trimoxazole Combination against Typhoidal Infectious Pathogens

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## Abstract

**Background:** Typhoidal infections pose significant public health concern on a global scale. Chloramphenicol and co-trimoxazole (trimethoprim-sulfamethoxazole) are both antibiotics used to treat typhoidal infections, often with co-trimoxazole considered a suitable alternative to chloramphenicol due to the potential serious side effects associated with chloramphenicol. But due to increasing reports over two decades on typhoidal infectious antibiotic resistance to these antibiotics, there necessitates exploration of alternative treatment strategies. This study investigated the *in vitro* antibacterial potency and synergistic effect of chloramphenicol and co-trimoxazole combination against *Salmonella typhi* (ATCC 334538).

**Method:** The antibacterial activity of chloramphenicol and co-trimoxazole, both alone and in combination at ratios of 1:1, 2:1, 4:1, 8:1, and 1:8 respectively, were assessed against *S. typhi*. Minimum Inhibitory Concentrations (MICs) were determined for each drug and in combination and were compared with their standard MICs according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The Fractional Inhibitory Concentration Index (FICI) was calculated to characterize the interaction as synergistic ( $FICI \leq 0.5$ ), additive ( $0.5 < FICI \leq 1$ ), indifferent ( $1 < FICI \leq 4$ ), or antagonistic ( $FICI > 4$ ). Clinical breakpoints were determined according to CLSI guidelines.

**Results:** The *Salmonella typhi* exhibited resistance to chloramphenicol and co-trimoxazole when used alone, with MICs of 64 µg/mL and 16 µg/mL, respectively. The combination of chloramphenicol and co-trimoxazole however significantly reduced the MICs, ranging from 2-16 µg/mL for chloramphenicol and 4-8 µg/mL for co-trimoxazole. Synergistic activity was observed with combination ratios of 4:1 and 8:1. Additive effects were seen with ratios of 1:1 and 2:1. A ratio of 1:8 demonstrated indifference. No antagonistic activity was detected.

**Conclusion:** These *in vitro* findings suggest that combining chloramphenicol and co-trimoxazole may be a relevant strategy for treating typhoidal infections, as synergistic combinations effectively lowered the MICs of both drugs against *S. typhi*. Further research, including *in vivo* studies and clinical trials, is warranted to evaluate the clinical efficacy and safety of this drug combination.

**Keywords:** Antibacterial synergy • Typhoid • Antibiotics • *Salmonella typhi* • Synergistic effects

## Introduction

Typhoidal infections pose significant public health concern on a global scale. It is caused by the invasion and manipulation of the two rod-shaped, gram-negative flagellated facultatively anaerobic bacilli *Salmonella enterica* serovar typhi (*S. typhi*) and paratyphi (*S. paratyphi*) A, B and C causing a life-threatening systemic illness called enteric fever. Humans are the exclusive host responsible for harboring both *S.*

*typhi* and *S. paratyphi* as reservoirs, with transmission typically occurring via the fecal-oral route, often through the consumption of contaminated food or water [1-3]. Following infection, the bacteria enter the bloodstream and the gastrointestinal system, resulting in symptoms such as persistent high fever, fatigue, headache, nausea, abdominal pains, and either constipation or diarrhea. In rare instances, a rash may also appear, and in severe cases, serious complications or fatal outcomes can occur [1,4].

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Typhoid and paratyphoid fevers stem from distinct yet related strains of *Salmonella* species, leading to similar symptomatology. Paratyphoid infections typically exhibit milder and less frequent manifestations compared to typhoid fever, with an average incubation period of 1 to 10 days. Conversely, the incubation period for typhoid fever typically ranges from 6 to 30 days [5-7].

Poor hygiene and sanitation standards in low and middle-income countries, including regions in the Middle East, some countries in Southern and Eastern Europe, parts of Central and South America, Africa and Asia, have contributed to the prevalence of typhoidal infections and resulted in elevated rates of morbidity and mortality [1,3,4].

Antibiotic treatment has been the cornerstone of managing typhoidal infections, with mortality rate as high as 30% in untreated cases but dropping to less than 1% with appropriate antibiotic therapy [8]. When infections are not properly managed and treated, patients may experience prolonged illness and a range of complications, including intestinal perforation, pneumonia, meningitis, endocarditis, osteomyelitis, and arthritis. This situation can increase the risk of individuals becoming carriers of the infection and spreading resistant strains to others [2,8].

Even though typhoidal infections can be effectively managed with antibiotics, the challenge lies in the evolving resistance patterns seen in strains of typhoidal *Salmonella* among drugs such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole, Multidrug-Resistant (MDR) [9]. One study showed 40 out of 51 *S. typhi* isolates were multidrug-resistant including chloramphenicol [10]. During 2012 to 2018 in Islamabad, Pakistan, a study confirmed *S. typhi* resistance among both chloramphenicol (46.9%) and co-trimoxazole (61.4%) [7]. With the emergence of MDR *Salmonella* isolates, ciprofloxacin, a fluoroquinolone, has since emerged as the drug of choice for the treatment of typhoidal infections. However, uncontrolled use of ciprofloxacin has led to increase resistance with one study reporting increase in *S. typhi* resistance against ciprofloxacin from 8.1% to 95% during 2016 to 2019 [11]. Following the widespread of ciprofloxacin resistant *S. typhi* isolates, ceftriaxone (third generation cephalosporin) and azithromycin (macrolide) has emerged as alternatives for the treatment of typhoidal infections. Although resistance of *S. typhi* and paratyphi remain rare with these drugs, sporadic cases have been reported with 13 azithromycin resistant *Salmonella* isolates identified in Bangladesh [12] and a steady increase in resistance from 0% in 2016, 3.2% in 2017 and 8.5% in 2018, another study reported [13]. Third generation cephalosporin resistant *Salmonella* strains have also been reported in various countries including Pakistan, Democratic Republic of Congo, Spain and Kuwait [14-16]. Based on the emerging resistance to the standard drugs, in this current study, we sought to investigate the combination therapy of antibiotics to ascertain their effectiveness.

Antimicrobial resistance poses a significant threat to human health, seriously jeopardizing our ability to combat infectious diseases. Generally, combination therapy has been shown to be an effective strategy in the fight against antimicrobial resistance leveraging a highly promising approach via the utilization of antimicrobial synergism. Antibacterial synergism, which is a sub-branch of antimicrobial synergism, is a phenomenon where the combined use of two or more antibiotic agents results in a greater effect than the sum of their individual effects [17]. In the context of typhoidal infections, the combination of chloramphenicol and co-trimoxazole has the potential to enhance the therapeutic arsenal against this challenging disease via the exploration of their synergistic mechanism of action.

Recently, there have been reports of re-emergence of conventional first line drugs-susceptible strains of *Salmonella* species in Nepal [18,19]. The susceptibility of *Salmonella typhi* to chloramphenicol and co-trimoxazole (trimethoprim-sulfamethoxazole) was 100%, while that to ampicillin was 98.2%. Similarly, all *S. paratyphi* A strains showed sensitivity to co-trimoxazole (trimethoprim-sulfamethoxazole) and ampicillin, with 96.7% of the isolates being sensitive to chloramphenicol [19]. Several other studies have also supported the findings on the re-emergence of the susceptibility of these conventional first-line drugs in *Salmonella typhi* and *paratyphi* isolates [20]. Nevertheless, the re-emergence and utilization of conventional first-line antibiotics in the treatment of typhoidal infections must adhere to rational practices to prevent the immediate reversal of *Salmonella* isolates exhibiting the classical Multidrug-Resistance (MDR) phenotype [18].

As the current study explores the synergistic effects of chloramphenicol and co-trimoxazole combination against typhoidal infectious pathogens, the knowledge on the efficacy of this antibacterial combination may lead to the development of more effective treatment approaches for typhoidal infections, potentially improving clinical outcomes, providing insights into alternative treatment options, reducing the burden of disease on patients and ultimately combating against antibiotic resistance, a global health threat that complicates the management of typhoidal infections [21].

## Materials and Methods

### Bacterial strains

The *Salmonella typhi* (ATCC 334538) isolates were used in the study and was obtained from the Microbiology Laboratory of the School of Pharmacy and Pharmaceutical Sciences of the University for Development Studies, Tamale, Ghana.

### Antibiotic samples and preparation

Chloramphenicol capsules BP 250 mg and co-trimoxazole tablets BP 80/400 mg were purchased from a pharmacy outlet in Kumasi, Ghana. 20 tablets of co-trimoxazole (480 mg) and 20 capsules of chloramphenicol (250 mg) were weighed using an analytical balance and

evaluated for their uniformity in weight according to the British Pharmacopoeia, 1953 specification. The drugs were powdered and stock solutions of 5000 µg/mL each of chloramphenicol and co-trimoxazole were prepared. Two-fold serial dilutions of each antibiotic stock solution were then prepared in Mueller-Hinton broth. Dilutions were designed to span a range encompassing both established clinical breakpoints and relevant therapeutic concentrations for each antibiotic, and included positive and negative controls. For the combination studies, chloramphenicol and co-trimoxazole were combined in 1:1, 2:1, 4:1, 8:1 and 1:8 ratios respectively.

### Antibacterial bioassay

The *Salmonella typhi* (ATCC 334538) isolates were sub-cultured in Mueller-Hinton Broth (MHB) and incubated overnight. Standardized bacterial suspensions, adjusted to 0.5 McFarland turbidity, were prepared in MHB. A broth microdilution assay was performed using 100 µL of MHB per well. Bacterial suspensions (20 µL) were added to each well, along with antibiotic dilutions (80 µL for single antibiotics, 40 µL each for antibiotic combinations). Microdilution trays included growth and sterility controls. Trays were incubated at 37°C for 24 hours. The Minimum Inhibitory Concentration (MIC) was visually determined as the lowest antibiotic concentration inhibiting visible growth. Minimum Bactericidal Concentrations (MBCs) were determined by sub-culturing 200 µL from MIC-containing wells onto Mueller-Hinton broth and identifying the lowest concentration yielding a ≥ 99.9% reduction in Colony Forming Units (CFUs)

### Synergy analysis (Determination of Fractional Inhibitory Concentration (FIC))

The combination of the chloramphenicol and co-trimoxazole interaction were analyzed using the standard checkerboard method [22]. The Fractional Inhibitory Concentration Index (FICI) was calculated according to the equation below:

$$FICI = \sum FIC = FIC_A + FIC_B = \left(\frac{C_A}{MIC_A}\right) + \left(\frac{C_B}{MIC_B}\right)$$

Antibiotic	MIC (µg/mL)	MBC (µg/mL)	Susceptibility	Activity
Chloramphenicol	64	512	Resistant	Bacteriostatic
Cotrimoxazole	16	64	Resistant	Bacteriostatic

**Table 1.** *In vitro* antibacterial activity of individual chloramphenicol and co-trimoxazole.

Table 2 presents the *in vitro* antibacterial activity of chloramphenicol and co-trimoxazole combination in various ratios (1:1, 2:1, 4:1, 8:1, and 1:8), along with their corresponding FICs. All combination ratios of chloramphenicol and co-trimoxazole led to a reduction in the MIC values of both antibiotics. Chloramphenicol MICs were reduced 4 to 32-fold, while co-trimoxazole MICs were reduced 1 to 8-fold compared to their individual MIC values. The FICI interpretative criteria, based on the Loewe additivity zero interaction theory, followed by this present study to determine the synergistic effect

Where, FIC<sub>A</sub> and FIC<sub>B</sub> are the fractional inhibitory concentrations of chloramphenicol and co-trimoxazole respectively, MIC<sub>A</sub> and MIC<sub>B</sub> are the MIC of chloramphenicol and cotrimoxazole alone, and C<sub>A</sub> and C<sub>B</sub> are the concentrations of chloramphenicol and co-trimoxazole in the combination in wells corresponding to the MICs. The FICI interpretative criteria were followed to determine the synergistic effect of the chloramphenicol and co-trimoxazole combination against the *Salmonella typhi* isolates: Antagonism (FICI ≥ 4), indifference (1 ≤ FICI < 4), additive (0.5 < FICI < 1) and synergism (FICI ≤ 0.5) [22]. This interpretation was based on the Loewe additivity zero interaction theory [23].

## Results and Discussion

The *in vitro* antibacterial activities of chloramphenicol and co-trimoxazole, both alone and in combination, against the *S. typhi* strain were conducted. Table 1 displays the MIC and MBC values for chloramphenicol and co-trimoxazole when tested individually. The MIC of chloramphenicol was 64 µg/mL, and the MBC was 512 µg/mL, resulting in an MBC/MIC ratio of 8. The MIC of co-trimoxazole was 16 µg/mL, and the MBC was 64 µg/mL, resulting in an MBC/MIC ratio of 4. The MIC values of chloramphenicol (64 µg/mL) was higher than the CLSI-defined resistance breakpoint (≥ 32 µg/mL), indicating resistance in the tested *S. typhi*. This finding aligns with previous reports documenting increasing chloramphenicol resistance in *S. typhi* [24,25] but in contrast to the studies by Adabara et al., Ahasan et al., Poudel et al., and Shrestha et al., which reported re-emergence of susceptibility to chloramphenicol [19,20,26-28]. The study by Donkor and colleagues agree with the findings of the study as co-trimoxazole was observed to be resistant against *S. typhi* [24]. However, this contrasted with the findings reported by Ahasan et al., and Chand et al., studies.

of the chloramphenicol and cotrimoxazole combination against the clinical isolates of *Salmonella typhi* were: Antagonism (FICI ≥ 4), indifference (1 ≤ FICI < 4), additive (0.5 < FICI < 1) and synergism (FICI ≤ 0.5) [22,23]. Two of the combined ratios of chloramphenicol and co-trimoxazole (1:1 and 2:1) gave FICI values of 0.625 and 0.75 respectively indicated that their combination proportions have additive effects (that is, the combined effect of both drugs is equal to the sum of their individual effects). The combination of chloramphenicol and co-

trimoxazole in 1:8 ratio produced an FICI of 1.031, indicating an indifference effect (that is, the combined effect of two drugs is neither greater nor less than the effect of each drug when used alone). Ultimately, the combination ratios 4:1 and 8:1 of chloramphenicol and

co-trimoxazole which gave FICI values of 0.5 and 0.375 respectively demonstrated synergistic effect (that is, the combined effect of both drugs is greater than the sum of their individual effects).

Antibiotic combination	Ratio ( $\mu\text{g/mL}/\mu\text{g/mL}$ )									
	1:1		2:1		4:1		8:1		1:8	
	Conc.	MIC	Conc.	MIC	Conc.	MIC	Conc.	MIC	Conc.	MIC
Chloramphenicol	16	8	32	16	32	16	32	16	4	2
Co-trimoxazole	16	8	16	8	8	4	4	2	32	16
FIC	0.625	0.75	0.5	0.375	1.031					
Interpretation	Additive	Additive	Synergism	Synergism	Indifference					

**Table 2.** *In vitro* antibacterial activity of chloramphenicol and co-trimoxazole combination at different proportions.

## Conclusion

In this study, chloramphenicol and co-trimoxazole, when tested individually *in vitro*, showed limited activity against *Salmonella typhi*, indicating resistance. This observation contrasts with recent *in vivo* reports that have demonstrated a positive reversal trend in susceptibility, with isolates responding favorably to these drugs. However, when chloramphenicol and co-trimoxazole were combined in 4:1 and 8:1 respectively, a marked synergistic antibacterial effect was observed against the *Salmonella typhi*. This promising *in vitro* assay findings lend credence to alternative strategy in combating against typhoidal infectious diseases posing great threat to global health amid fast-rising antimicrobial resistance. Further *in vivo* studies are needed to clinically validate the synergistic interaction of chloramphenicol and co-trimoxazole in the right combination ratio to enhance treatment efficacy and overcome resistance development in *S. typhi* infections.

## Competing Interests

The authors declare no conflict of interest in relation to this work.

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## Authors' Contributions

AY and LCN conceived and designed the study; LCN performed the experiments; AY and LCN wrote the paper. All authors have read and approved the final version of the manuscript.

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