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Research Article

EVALUATION OF THE ROLE OF ANTIOXIDANT VITAMIN C IN REVERSAL OF ANTIMICROBIAL AGENT (AMOXICILLIN) RESISTANCE

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ABSTRACT

Objectives: To evaluate the potential of Vitamin C in reversing microbial resistance to amoxicillin in *Escherichia coli* strains (CFT073) by exploring its antioxidant property as a modulator of antibiotic efficacy.

Methods: An *in vitro* study was conducted using amoxicillin-resistant *E. coli* (CFT073) isolates. The antimicrobial effects of amoxicillin alone, Vitamin C alone (5 mg/mL and 10 mg/mL), and combinations of amoxicillin with Vitamin C were tested. The zone of inhibition was measured on Day 1, Day 3, and Day 5 to assess antibacterial activity. Statistical analysis was performed using Student's t-test.

Results: Vitamin C alone, at both concentrations, did not exhibit any inhibitory effect. Amoxicillin alone did not produce inhibition. The combination of amoxicillin with Vitamin C at 5 mg/mL and 10 mg/mL enhanced the antibacterial activity significantly, with zones of inhibition measuring 14.8±0.92 mm and 16.2±0.93 mm, respectively ($p<0.05$).

Conclusion: Vitamin C, when combined with amoxicillin, significantly increased the susceptibility of resistant *E. coli* strains by potentially reducing oxidative stress. Vitamin C, being safe, inexpensive, and widely available, could serve as an effective adjuvant in antimicrobial therapy.

Keywords: Vitamin C, Amoxicillin, Antimicrobial resistance, *Escherichia coli*, Oxidative stress, Adjuvant therapy.

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INTRODUCTION

The widespread use of antimicrobial agents has considerably reduced the burden of infectious diseases; however, the escalating challenge of antimicrobial resistance (AMR) has emerged as a global health crisis, threatening the efficacy of standard antibiotic therapies [1]. Among the mechanisms underlying resistance, the capacity of bacteria to evade commonly used antibiotics like amoxicillin plays a central role [1]. This resistance is frequently linked to oxidative stress, which not only accelerates genetic [1] mutations but also facilitates bacterial survival under antibiotic pressure [1].

Vitamin C (ascorbic acid), recognized for its potent antioxidant and anti-inflammatory properties [2], has recently drawn scientific interest for its potential in mitigating AMR. It exerts its effects primarily by scavenging reactive oxygen species, thereby modulating oxidative stress pathways that contribute to resistance development [2]. Emerging evidence indicates that Vitamin C exhibits significant antibacterial and anti-biofilm activity against a range of drug-resistant pathogens, including *Pseudomonas aeruginosa* and uropathogenic *Escherichia coli* [3,4]. In addition, its capacity to disrupt biofilm architecture and enhance bacterial susceptibility to antibiotics further underscores its therapeutic promise [5].

This study was undertaken to evaluate the role of the antioxidant property of Vitamin C in reversing microbial resistance to amoxicillin in *E. coli* strains (CFT073). By exploring this novel combination, the study aims to provide insights into potential strategies to combat antibiotic resistance and enhance the efficacy of existing antimicrobial agents.

METHODS

Study design

This was an *in vitro* experimental study designed to evaluate the effect of Vitamin C in combination with amoxicillin on amoxicillin-resistant *E. coli* (CFT073) isolates.

Bacterial strain

The bacterial strain used in this study was a standard amoxicillin-resistant *E. coli* (CFT073), obtained from the urinary tract infection samples in the microbiology laboratory. All bacterial cultures were maintained on nutrient agar under appropriate conditions.

Study groups

Six experimental groups were included:

- Culture media alone (control)
- Amoxicillin alone
- Vitamin C (5 mg/mL) alone
- Amoxicillin + Vitamin C (5 mg/mL)
- Vitamin C (10 mg/mL) alone
- Amoxicillin + Vitamin C (10 mg/mL).

Preparation of discs and inoculation

Sterile discs were impregnated with the respective test substances and placed on nutrient agar plates pre-inoculated with *E. coli* cultures. Plates were incubated under standard aerobic conditions.

Assessment of antibacterial activity

The antibacterial activity was assessed using the disc diffusion method. The zone of inhibition around each disc was measured in millimeters

(mm) using a standard measuring scale. Measurements were taken at 3 time points: Day 1, Day 3, and Day 5.

Data collection and analysis

All experiments were performed in triplicate to ensure accuracy and reproducibility. The mean zone of inhibition and standard deviation were calculated for each group and time point.

Statistical analysis

Data were analyzed using Student's t-test to compare differences between the groups. $p<0.05$ was considered statistically significant.

RESULTS

The present study evaluated the effect of Vitamin C in combination with amoxicillin on amoxicillin-resistant *E. coli* (CFT073), using the zone of inhibition as a marker of antibacterial activity. The antimicrobial activity of various treatment groups was assessed over 3 time points: Day 1, Day 3, and Day 5.

The culture media alone, amoxicillin alone, and Vitamin C alone at both concentrations (5 mg/mL and 10 mg/mL) did not demonstrate any inhibitory effect on bacterial growth at any time point, with the zone of inhibition consistently measuring 0.0 mm (Table 1).

Combination of amoxicillin with Vitamin C at concentrations of 5 mg/mL produced a notable increase in bacterial inhibition over time, with the zone of inhibition increasing from 7.6±0.56 mm on Day 1 to 9.4±0.89 mm on Day 3, and reaching 14.8±0.92 mm on Day 5 and Combination of amoxicillin with Vitamin C 10 mg/mL showed remarkable bacterial inhibition over time, with the zone of inhibition increasing from 9.6±0.47 mm on Day 1 to 11.7±0.68 mm on Day 3, and reaching 16.2±0.93 mm on Day 5 (Table 1).

The combination of amoxicillin with Vitamin C resulted in a significant enhancement of antibacterial activity. The addition of Vitamin C at 5 mg/mL to amoxicillin produced a zone of inhibition of 14.8±0.92 mm on Day 5, which was significantly higher compared to earlier time points ($p<0.05$). Similarly, the combination of amoxicillin with Vitamin C at 10 mg/mL exhibited the largest zone of inhibition of 16.2±0.93 mm on Day 5, which was statistically significant when compared to amoxicillin alone ($p<0.05$) (Table 2).

The summary of the antimicrobial effects of each treatment group is presented in Table 3.

The results highlight that while Vitamin C alone did not exhibit any antimicrobial activity, its combination with amoxicillin enhanced the susceptibility of resistant *E. coli* to the antibiotic, with the 10 mg/mL concentration yielding the greatest inhibitory effect. These findings suggest a potential role for Vitamin C as an adjuvant in antimicrobial therapy to combat bacterial resistance.

Table 1: Zone of inhibition (mm) for different groups over time

Group	Day 1 (mm)	Day 3 (mm)	Day 5 (mm)
Culture media alone	0.0±0.0	0.0±0.0	0.0±0.0
Amoxicillin	0.0±0.0	0.0±0.0	0.0±0.0
Vitamin C (5 mg/mL)	0.0±0.0	0.0±0.0	0.0±0.0
Amoxicillin+Vitamin C (5 mg/mL)	7.6±0.56	9.4±0.89	14.8±0.92*
Vitamin C (10 mg/mL)	0.0±0.0	0.0±0.0	0.0±0.0
Amoxicillin+Vitamin C (10 mg/mL)	9.6±0.47	11.7±0.68	16.2±0.93*

*Statistically significant difference on Day 5 compared to Day 1 and Day 3 ($p<0.05$)

Table 2: Comparison of zone of inhibition on day 5

Group	Zone of inhibition (mm) on Day 5	p-value
Amoxicillin Alone	0.0±0.0	Reference
Amoxicillin+Vitamin C (5 mg/mL)	14.8±0.92	<0.05*
Amoxicillin+Vitamin C (10 mg/mL)	16.2±0.93	<0.05*

*Significant improvement in bacterial inhibition with Vitamin C combination

Table 3: Summary of observations for different treatments

Treatment	Effect on *Escherichia coli* resistance	Zone of inhibition observed
Culture media alone	No inhibition	0.0 mm
Vitamin C (5 mg/mL) Alone	No inhibition	0.0 mm
Vitamin C (10 mg/mL) Alone	No inhibition	0.0 mm
Amoxicillin alone	No inhibition	0.0 mm
Amoxicillin+Vitamin C (5 mg/mL)	Significant enhancement of amoxicillin activity	14.8 mm (Day 5)
Amoxicillin+Vitamin C (10 mg/mL)	Maximum enhancement of amoxicillin activity	16.2 mm (Day 5)

DISCUSSION

The present study demonstrates that the combination of Vitamin C with amoxicillin significantly enhances antibacterial efficacy against amoxicillin-resistant *E. coli* (CFT073). While amoxicillin alone failed to produce any inhibitory effect over the 5-day observation period, the co-administration of Vitamin C, particularly at a concentration of 10 mg/mL, resulted in a statistically significant increase in the zone of inhibition, indicating a potentiating effect of Vitamin C on antibiotic action. These findings are in line with previous *in vitro* and *in vivo* studies that support the synergistic potential of Vitamin C when used alongside antibiotics against resistant bacterial strains [6,7].

Vitamin C, a well-established antioxidant, mitigates oxidative stress by scavenging reactive oxygen species (ROS), which are implicated in bacterial mutagenesis and resistance development. This antioxidant mechanism may explain its ability to restore or enhance bacterial susceptibility to antibiotics [8]. Importantly, the current study observed no direct antibacterial effect of Vitamin C alone, reinforcing its adjuvant role rather than that of a standalone antimicrobial agent. This observation concurs with prior findings demonstrating that Vitamin C primarily functions as a resistance-modifying agent rather than exerting direct bactericidal effects [8,9].

The dose-dependent nature of this enhancement was evident, as the higher concentration of Vitamin C (10 mg/mL) resulted in a more pronounced antibacterial effect compared to the 5 mg/mL dose. Such a relationship has been previously reported, where escalating concentrations of Vitamin C improved antimicrobial outcomes, especially against multidrug-resistant pathogens [7,10]. This finding holds clinical relevance, suggesting that optimizing Vitamin C dosing could potentiate antibiotic regimens and reduce reliance on high-dose antimicrobials.

Furthermore, Vitamin C has been shown to alter biofilm dynamics and impair virulence factors in various resistant organisms, including *Klebsiella pneumoniae* and *Staphylococcus aureus*, suggesting broader implications beyond *E. coli* alone [9,10]. The capacity of Vitamin C to enhance antibiotic penetration through biofilm disruption further supports its clinical utility as an adjunctive therapy in resistant infections [8-10].

CONCLUSION

The present study demonstrates that the addition of Vitamin C significantly enhances the antibacterial activity of amoxicillin against amoxicillin-resistant *E. coli* (CFT073).in vitro. While Vitamin C alone

showed no direct antimicrobial effect, its combination with amoxicillin produced a greater zone of inhibition, particularly at a higher concentration (10 mg/mL), suggesting a potential dose-dependent synergistic effect. The antioxidant properties of Vitamin C may help reverse bacterial resistance by reducing oxidative stress, thereby restoring antibiotic efficacy. Given its safety, affordability, and accessibility, Vitamin C could serve as a promising adjuvant in antimicrobial therapy. Further *in vivo* and clinical studies are warranted to confirm these findings and explore clinical applications.

AUTHOR CONTRIBUTION

RTD- Concept and design of the study, results interpretation, review of literature, and preparing the first draft of the manuscript. Statistical analysis and interpretation, revision of manuscript. AN- Concept and design of the study, results interpretation, review of literature, and preparing the first draft of the manuscript, and revision of the manuscript. AS- Results interpretation, review of literature, and preparing the first draft of the manuscript. Statistical analysis and interpretation. SIM- Review of literature and preparing the first draft of the manuscript. Statistical analysis and interpretation

CONFLICT OF INTEREST

No conflict of interest is associated with this research.

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