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RESEARCH ARTICLE

Anti-Bacterial Activity of Levofloxacin alone and in Combination with Cetirizine Dihydrochloride Against *Escherichia Coli* and Methicillin-Resistant *Staphylococcus Aureus* (MRSA)

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ABSTRACT

The rise of antibiotic resistance has created an urgent need for novel therapeutic strategies against multidrug-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA). Levofloxacin, a widely used fluoroquinolone, faces reduced efficacy due to bacterial resistance. Combining levofloxacin with non-antibiotic agents, such as cetirizine dihydrochloride, may enhance antibacterial activity and help overcome resistance. This study explores the synergistic effects of levofloxacin and cetirizine as a potential treatment against *E. coli* and *S. aureus*. Fifty-five clinical isolates were collected from Mayo Hospital, Lahore, Pakistan. Bacterial identification was performed using classical, biochemical, and PCR techniques. Antimicrobial susceptibility was assessed via disc diffusion and well diffusion methods, while MIC values were determined using the broth microdilution method. Synergy was evaluated through the checkerboard assay, and DNA damage in lymphocytes was analyzed using the comet assay. Out of fifty-five, 17 *S. aureus* and 11 *E. coli* were found positive. The combination of levofloxacin and cetirizine exhibited strong antibacterial activity, with MIC values of 8 µg/mL for *S. aureus* and 2 µg/mL for *E. coli*. PCR confirmed the presence of the *Iss* (323 bp) and *nuc* (450 bp) genes in *E. coli* and *S. aureus*, respectively. The comet assay revealed no significant genotoxic effects at therapeutic concentrations. The synergistic combination of levofloxacin and cetirizine demonstrated potent antibacterial effects against *E. coli* and *S. aureus* without inducing genotoxicity. This novel dual-drug approach presents a promising strategy for combating multidrug-resistant bacterial infections.

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INTRODUCTION

Infectious diseases caused significant rates of illness and death globally before the 20th century. However, efforts to combat, treat, and control the spread of contagious diseases remained largely ineffective for an extended period due to a lack of knowledge and information (Mohr, 2016). Antibiotics presented a remarkable prospect for enhancing the quality of life

through the prevention of bacterial infections (Uddin *et al*., 2021). One of the most significant opportunistic bacterial infections in humans is caused by *S. aureus*, with approximately 20–30% of individuals experiencing persistent colonization in the nose. Additionally, frequent colonization of the skin, throat, axillae, groin, and intestine is common. This bacterium can lead to various types of infections, including acute, recurring, or chronic and persistent *S. aureus* infections (Hardy *et al*.,2020). A Gram (+) pathogen, Staphylococcus aureus can infect the respiratory system, soft tissues, skin, and bloodstream. It is among the primary causes of community and nosocomial infections (Carmona-Orozco, 2024)

Reports indicate that *S. aureus* widely affects clinical samples from both newborns and adults suffering from various respiratory conditions, including COPD, cystic fibrosis, community-acquired pneumonia, and hospital-acquired respiratory infections (Defres *et al*., 2009; Goss and Muhlebach, 2011; Stefani *et al*., 2019). Lower respiratory tract infections are mainly attributed to Gram-negative bacteria (GNB), like Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and other Enterobacteriaceae species. These organisms are major contributors to bacterial resistance, particularly in the form of multidrug-resistant (MDR) GNB (Rodrigo-Troyano and Sibila, 2017; Azuma *et al*., 2019; Badr *et al*., 2022).

Fluoroquinolones actively combat a wide range of bacteria, proving effective against both Gram-positive and Gram-negative strains (Hooper and Jacoby, 2015; Baggio and Ananda-Rajah, 2021). Levofloxacin, being a third-generation fluoroquinolone, exerts its antibacterial effects by inhibiting DNA synthesis. This mechanism allows it to demonstrate activity against Staphylococcus aureus and exhibit high antibacterial efficacy in vitro, particularly against many members of the Enterobacteriaceae family (Wronska *et al*., 2019; Mansouri *et al*., 2022). Levofloxacin, an FDA-approved antibiotic, has a wide range of indications for various infections. Doctors use it to treat hospital-acquired pneumonia, acute bacterial rhinosinusitis, acute bacterial prostatitis, acute pyelonephritis, urinary tract infections, community-acquired pneumonia, and skin or soft tissue infections. Additionally, it is utilized for Yersinia pestis plaque prophylaxis and treatment, and in certain situations, it is employed to reduce the incidence of infections. Levofloxacin belongs to the fluoroquinolone family of antibiotics (Podder and Sadiq, 2019). Several factors may induce resistance to quinolones, including the inactivation of the DNA gyrase enzyme, reduced cell membrane permeability, and the presence of active efflux pumps. These factors can act alone or in combination to confer resistance to quinolones (Sierra *et al*., 2005).

The potential combinational effect of antihistamines and antibacterial agents holds promise as a viable option for treating infectious diseases in the future. This approach could not only enhance the therapeutic efficacy but also offer the opportunity to reduce the overall number of antibacterial agents used. By harnessing the synergy between antihistamines and antibiotics, this innovative strategy may contribute to more effective and efficient management of infectious diseases, providing potential benefits for patients and public health.Bruer *et al*., 2019).

Histamine is a chemical that is released from mast cells and interacts with cells that have histamine receptors. Mast cells, which are abundantly present in the respiratory tree, gastrointestinal tract, and skin, possess high concentrations of histamine. When histamine binds to these cells, it triggers their activation and leads to the release of other chemicals, ultimately causing allergy symptoms (Salem *et al*., 2022). The exact mechanism by which antihistamines exhibit antibacterial activity has not been fully elucidated, but there are various theories. One concept suggests that antihistamines may alter the permeability of bacterial membranes, leading to their antibacterial effects against *S. aureus* and *E. coli*. These compounds demonstrate good absorption from the bacterial cell surface, which contributes to their antibacterial properties. But further investigation is required to completely comprehend and characterize the specific mechanisms involved in the antibacterial action of antihistamines (Lagadinou *et al*., 2020).

Antibacterial resistance is an escalating threat, and unfortunately, the discovery of new therapeutics is currently restricted. This calls for urgent research and development efforts to combat the rising challenges posed by resistant bacteria and ensure effective treatments for infectious diseases in the future. Consequently, existing antimicrobial drugs may not provide adequate antibacterial therapy and can lead to increased adverse effects. This study provides insights into combination therapy, where antibiotics are used in combination with non-antibiotic agents, as a potential strategy to overcome acquired and intrinsic resistance.

This study aims to investigate the synergistic effects of levofloxacin, both alone and in combination with cetirizine dihydrochloride, against *Escherichia coli* and *Staphylococcus aureus*. This combination holds potential for improving antibacterial efficacy,

enhancing safety, and mitigating levofloxacin resistance.

MATERIALS AND METHODS

Drugs: The active ingredients of cetirizine dihydrochloride and levofloxacin used in this study were obtained from RASCO Pharmaceuticals, located in Lahore, Pakistan. Additionally, other chemicals, including methanol, were sourced from Sigma-Aldrich, based in Germany. Researchers used Mueller–Hinton agar, nutrient agar, and Staph-110 agar as culture media, which were acquired from High Media Labs, India.

Microorganisms: A total of 55 clinical samples were collected from patients at Mayo Hospital in Lahore, Pakistan. The samples were obtained using transport swabs containing Amies transport medium, following the Clinical and Laboratory Standards Institute's (CLSI) safety recommendations. Only specimens that tested negative for *Mycobacterium tuberculosis* were stored. Of the 55 clinical isolates, 17 isolates of *S. aureus* and 11 isolates of *E. coli* were identified using normal visual and biochemical techniques, as well as polymerase chain reaction (PCR) (Ali *et al*., 2014; Haq *et al*., 2022). Ethical approval for this study was obtained from the Institutional Review Committee of the University of Veterinary and Animal Sciences (UVAS), Lahore, under application number 325/IRC/BMR. Informed consent was obtained from all participants before sample collection.

Preparation of culture media: The main culture was grown on nutrient agar. To subculture *E. coli* from the initial culture, the cells were grown on EMB agar (Mirani, 2018). The *S. aureus* cells were subcultured on Staph 110 agar. Gram staining was applied for identification. After coloring, the glass slides were viewed under a light microscope at a magnification of 100X (Cheesbrough, 2005).

Biochemical characterization of *S. aureus* and *E. coli:* To describe *S. aureus*, the catalase test, coagulase test, and mannitol fermentation test were run. The identification of *E. coli* was carried out using the indole, Methyl red, and catalase tests (Atala and Aldabagh, 2017).

DNA extraction: DNA was extracted from single CFUs of 11 *E. coli* and 17 *S. aureus* isolates using the Qiagen DNA tissue/blood/bacteria isolation kit. The DNA concentration was quantified using Nanodrop, and gel electrophoresis on a 0.5% agarose gel was performed to verify integrity.

Primer design for *E. coli* and *S. aureus:* In this study, we identified the primer sequences for resistance gene identification in *E. coli* and *S. aureus* from the NCBI database. Subsequently, we designed the primers using the online tool Primer3. Detailed information on the primer sequences for *E. coli* and *Staphylococcus aureus* can be found in Tables 1 and 2, respectively.

Table 1: Molecular Identification of *S. aureus*: A Comprehensive Guide to Primers

Target gene	Product Size (bp)	Primer Sequence (5'-3')
Nuc	450	F - AGTATATAGTGCAACTTCAACTAAA
		R - ATCAGCGTTGTCTTCGCTCCAAATA

Table 2: Molecular Identification of *E. coli:* Exploring the Targeted Primers

Target gene	Product Size (bp)	Primer Sequence (5'-3')
Iss	323	F- CAGCAACCCGAACCACTTGATG
		R - AGCATTGCCAGAGCGGCAGAA

PCR amplification of *E. coli* and *S. aureus*: PCR amplification was conducted separately for *E. coli* and *S. aureus*. The target DNA was mixed with PCR master mix, primers, and PCR-grade water, and amplified in a thermocycler with specific cycling conditions. Each bacterium grew for approximately 45 minutes.

Disc diffusion method: Eleven *E. Coli* isolates and seventeen MRSA isolates each underwent an antimicrobial susceptibility test using the disc diffusion method (Kirby-Bauer technique) (Hudzicki, 2009). A bacterial solution with a standard concentration of 1.5 × 108 CFU (0.5 McFarland) was used. The sensitivity was assessed using many antibiotic discs, such as those containing levofloxacin (5 µg) and (30 µg). The CLSI standards were adhered to as the protocol for this surgery (Hsueh *et al*., 2010).

Well diffusion method: Mueller-Hinton agar was made and sterilized in an exact quantity. Using a sterilized glass puncher, 6-mm diameter wells were punched, and agarose gel agar was used to seal the well bases. The standardized bacterial solution (0.5 McFarland) was tested against cetirizine dihydrochloride and levofloxacin at increasing concentrations (multiples of two, i.e., 1, 2, 4 1024 µg/ml) Banoee *et al*. (2010).

MIC determination: Using the broth microdilution technique, the MICs of 11 E. coli isolates and 17 clinical isolates of MRSA were determined (Koeth *et al*., 2023) . Ten dilutions ranging from 1024-1 µg/ml were prepared by twice diluting the stock concentration of medicines to determine the minimum inhibitory concentration (MIC). The resistance breakpoints of the medicines were matched with the British Society for Antimicrobial Chemotherapy recommendations (Churchill *et al*., 2020) .

Synergy test by checkerboard method and fractional inhibitory concentration index: Both bacteria's MICs for cetirizine dihydrochloride alone and in combination with levofloxacin were determined. Different dilutions were serially diluted vertically and horizontally, then spread out in a checkerboard pattern throughout the 96-well plate to determine the combined MIC of both medicines. Following a comparison of the measured value of the medication combination with the provided standard, the medicines' interactions were classified as antagonistic, neutral, or synergistic according to their fractional inhibitory concentration index (FICI) (Orhan *et al*., 2005)..

Comet assay: This study evaluated whether the combination of levofloxacin and cetirizine could overcome resistance. To ensure its safety, we tested its toxicity on lymphocytes (a type of white blood cell). A comet assay was used to check for any harmful effects. The goal was to confirm that the combination is both effective and non-toxic for use. Base slides were prepared by cleaning and filling cavity slides with agarose solution, and refrigerating them overnight. Blood (5 mL) was collected from a healthy sheep in a heparinized tube. Lymphocytes were separated from the blood using lymphocyte separating medium (Lymphocyte Separation Medium, Density 1.077 g/ml, Capricorn Scientific), and RPMI 1640 medium was added. Lymphocyte counting was performed using a hemocytometer. Test chemicals (levofloxacin: cetirizine) at varying doses (1024, 512, 256, 128, 64, 32, 16, 8µg/ml) were mixed with 100µl lymphocyte cell suspension and incubated. The evaluation of DNA damage involved layering low-melting agarose on slides, treatment with lysing and alkaline buffer solutions, electrophoresis, neutralization, staining with ethidium bromide, examination under a fluorescent microscope, and comet scoring using Comet IV software. Slides were stored, hardened, dipped, electrophoresed, neutralized, stained, examined, and scored to assess DNA damage (Moller *et al*., 2020).

RESULTS

Out of the total 55 clinical isolates, only 11 (20%) were identified as positive for *E. coli*, while 17 (30.9%) were positive for *S. aureus*. Among the 24 samples taken from patients with lower respiratory tract infections and nosocomial infections, 6 (25%) clinical isolates were positive for *E. coli*, and 11 (45.8%) were positive for MRSA. Additionally, 10 samples collected from patients with various diseases showed 4 (40%) positive isolates for *E. coli* and 6 (60%) positive isolates for MRSA.

Furthermore, 13 samples obtained from patients with seasonal cough and allergies had only 1 isolate (8.33%) testing positive for *E. coli*, while 7 (58.33%) isolates were positive for MRSA. Lastly, among the eight sputum samples taken from bronchitis patients, only 1 isolate (14.28%) was positive for *E. coli*, and 3 (42.85%) were positive for MRSA.

Identification and biochemical characterization of *S. aureus* and *E. coli*: Gram-staining revealed rounded, deeply purple Gram-positive bacteria, which helped to define *S. aureus*. The presence of *S. aureus* was confirmed by positive results from the mannitol fermentation test, coagulase test, and catalase test. Similarly, the presence of Gram-negative *E. coli* was established by thin, rod-shaped bacteria that were dyed pink. The presence of *E. coli* was also verified by positive results from the Indole and Methyl Red tests.

PCR identification of *E. coli*: The PCR amplification successfully validated the presence of a resistance gene within the *E. coli* bacteria. Specifically, the gene identified was the *Iss* gene, which was detected at the expected size of 323 base pairs.

PCR identification of *S. aureus*: The PCR amplification has provided conclusive evidence for the detection of a resistance gene in *S. aureus*. To be more specific, the gene identified is known as the *nuc* gene, and it was found to be present at the expected size of 450 base pairs.

Resistance Patterns of MRSA and *E. coli* Isolates: The resistance patterns of 17 MRSA isolates and 11 *E. coli* isolates were assessed following CLSI guidelines. Based on the zone of inhibition diameter, the isolates were classified as sensitive, intermediate, or resistant to antibiotics. MRSA showed high resistance to Penicillin G (98%), Augmentin (86%), and cefuroxime (83%), with 80% resistance to levofloxacin. *E. coli* isolates exhibited 80-90% resistance to Penicillin G, Cefuroxime, and Augmentin, and 70% resistance to levofloxacin (Tables 3 and 4).

Antibacterial susceptibility of cetirizine and levofloxacin: Fig. 1 and Fig. 2 present the susceptibility patterns of cetirizine and levofloxacin against *S. aureus* and *E. coli*, respectively. Only one dilution of cetirizine (512 µg) demonstrated sensitivity against both *S. aureus* and *E. coli*. In contrast, levofloxacin exhibited a distinct and effective zone, indicating sensitivity against both bacterial strains.

MIC: The MIC of cetirizine alone against different samples of *E. coli* and *S. aureus* was observed to be 512µg/ml. The MIC of levofloxacin alone against different samples of *E. coli* and *S. aureus* was observed to be different.

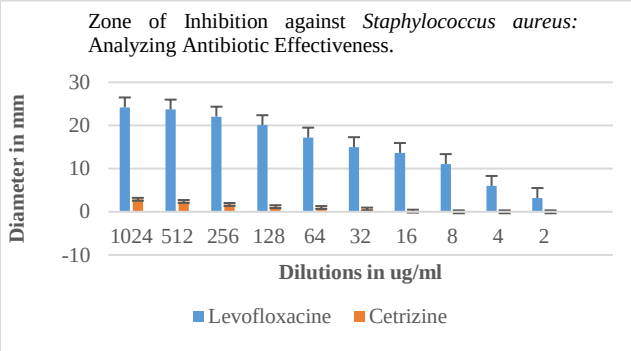

Fig 1: Comparison of mean zone of inhibition diameters of levofloxacin versus cetirizine against Staphylococcus aureus at a level of significance. ***p<0.005.

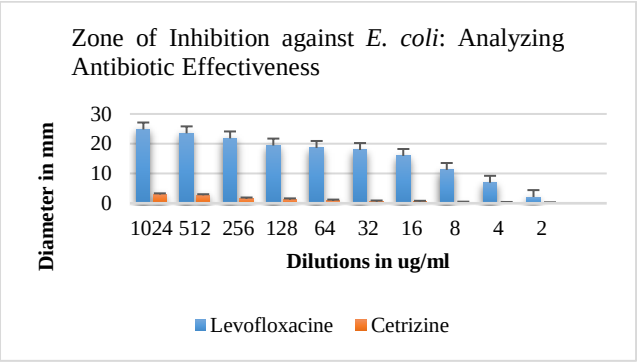

Fig 2: Comparison of mean zone of inhibition diameters of levofloxacin versus cetirizine against *E. coli* at a level of significance. ***p<0.005

FICI by checkerboard method: It was discovered that several bacterial samples at various MICs were "synergistic" in accordance with the FICI criteria. The mean combination of 8/512µg/ml Levofloxacin (drug A) and Cetirizine (drug B), respectively. The synergistic impact is maximized in the present investigation. As seen in Tables 5 and 6, the combination of 2/512µg/ml of drugs A and B produced the greatest synergism against *E. coli*. The findings are shown with a FICI of less than 0.5.

Comet assay: To evaluate the genotoxic effect of a combination, the comet assay was conducted. Different concentrations of levofloxacin and cetirizine (1024 µg/ml, 512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, and 8 µg/ml) were used, with the positive control being 20% DMSO and the negative control being PBS. No damage was observed at the concentration of 8 µg/ml. At the highest concentration of 1024 µg/ml, the mean tail length was 2.97±0.054, indicating less damage compared to the positive control (20% DMSO) with a mean tail length of 11.09±0.06. The genetic damage index at the very high dose of 1024 µg/ml was 0.48. It was found that the combination did not exhibit a genotoxic effect. DNA damage index is shown in Fig. 3 and Fig. 4.

Table 3: Interpretive zone diameter breakpoints for disk diffusion susceptibility testing for penicillin G, Augmentin, Gentamicin, levofloxacin and vancomycin against methicillin-resistant *Staphylococcus aureus* established by the Clinical and Laboratory Standards Institute.

Antibiotics	Interpretive zone diameters (mm) of resistance breakpoints of MRSA.			Resistance patterns of MRSA observed from clinical isolates according to zone diameter (mm).			
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Total isolates tested
Penicillin G	29	–	28	0	3	13	17
Gentamicin	15	13-14	12	11	5	1	17
Chloramphenicol	25	–	24	4	3	10	17
Cefuroxime	25	–	19	1	4	12	17
Augmentin	–	–	–	0	5	12	17
Vancomycin	–	–	–	16	1	0	17
Levofloxacin	19	16-18	15	2	4	11	17

Table 4: Interpretive zone diameter breakpoints for disk diffusion susceptibility testing for penicillin G, Augmentin, Gentamicin, levofloxacin and vancomycin against Escherichia coli established by the Clinical and Laboratory Standards Institute.

Antibiotics	Interpretive zone diameters (mm) of resistance breakpoints of MRSA.			Resistance patterns of *E. coli* observed from clinical isolates according to zone diameter (mm).			
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Total isolates tested
Penicillin G	29	–	28	0	2	9	11
Augmentin	15	13-14	12	0	3	8	11
Cefuroxime	25	–	24	1	3	7	11
Tigecycline	25	–	19	2	3	6	11
Levofloxacin	–	–	–	2	4	5	11
Gentamicin	–	–	–	1	4	6	11
Ceftriaxone	19	16-18	15	7	3	1	11

Table 5: FICI of Levofloxacin and Cetirizine 2HCL against Different Mean Samples of *S. aureus*

MIC µg/ml (combination)	MIC CET	MIC LEVO	FIC Drug CET	FIC Drug LEVO	FICI	Interpretation
1	8	8	0.125	0.125	0.253	Synergism
1	16	8	0.0625	0.125	0.187	Synergism
1	32	8	0.03125	0.125	0.156	Synergism
1	64	8	0.01562	0.125	0.1406	Synergism
1	128	8	0.00781	0.125	0.1328	Synergism
1	256	8	0.00390	0.125	0.1288	Synergism
1	512	8	0.0019	0.125	0.126	Synergism
1	1024	8	0.00097	0.125	0.115	Synergism

Table 6: FICI of Levofloxacin and Cetirizine 2HCL against Different Mean Samples of *E. coli*

MIC µg/ml (combination)	MIC CET	MIC LEVO	FIC Drug CET	FIC Drug LEVO	FICI	Interpretation

0.5	2	2	0.25	0.25	0.501	Synergism
0.5	4	2	0.125	0.25	0.375	Synergism
0.5	8	2	0.0625	0.25	0.312	Synergism
0.5	16	2	0.03125	0.25	0.2812	Synergism
0.5	32	2	0.0152	0.25	0.265	Synergism
0.5	64	2	0.007812	0.25	0.257	Synergism
0.5	128	2	0.0039	0.25	0.2539	Synergism
0.5	256	2	0.00195	0.25	0.2512	Synergism
0.5	512	2	0.000976	0.25	0.2415	Synergism
0.5	1024	2	0.000488	0.25	0.2143	Synergism

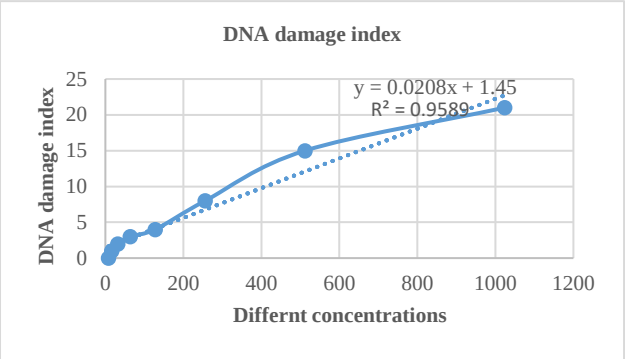

Fig. 3: Assessing the Genotoxic Potential of Levofloxacin and Cetirizine Combination

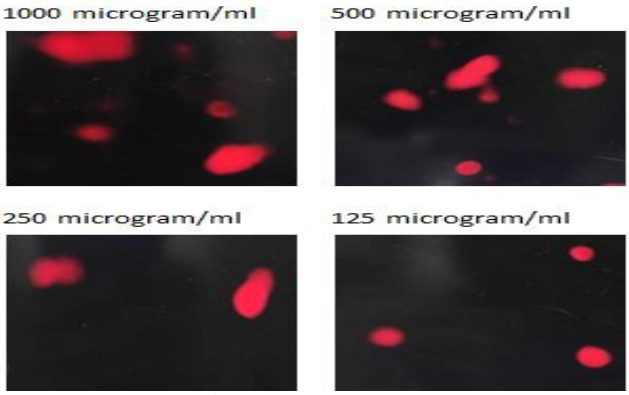

Fig. 4: DNA damage of lymphocytes under a Fluorescent Microscope with different drug concentrations.

DISCUSSION

Pathogenic bacterial infections remain a major health concern despite significant advancements in antibiotics, vaccines, and infection control (Bjarnsholt, 2013). Historically, antibiotics were considered the most effective means to combat bacterial infections. However, their effectiveness has declined due to widespread misuse, leading to the emergence of multidrug-resistant (MDR) strains (Wimmerstedt *et al*., 2008; Dugassa *et al*., 2017; Mancuso *et al*., 2021). Resistance mechanisms in bacteria include reduced uptake, efflux pumps, enzymatic degradation, and altered target sites (Fluit *et al*.,2001).

Fluoroquinolone use has contributed to resistance development in *P. aeruginosa*, *S. aureus*, and *E. coli* (MacDougall *et al*., 2005). Recent research highlights the potential of antihistamines, such as cetirizine, to enhance antibiotic efficacy by inhibiting efflux pumps (Gocmen *et al*., 2009; Bruer *et al*., 2019). This study assessed the effectiveness of levofloxacin alone and in combination with cetirizine against *E. coli* and *S. aureus*.

Of the 55 bacterial isolates examined, 17 *S. aureus* and 11 *E. coli* were confirmed via biochemical and Gram staining methods (Ali *et al*., 2014; Atala and Aldabagh, 2017). Disc diffusion revealed high resistance in both species, particularly against penicillin G and amoxicillin-clavulanate, while gentamicin and vancomycin remained effective (Gilbert *et al*., 2001; Kumar *et al*., 2011). *E. coli* was also resistant to cefuroxime but susceptible to ceftriaxone (Mos *et al*., 2010; Wu *et al*., 2016).

Resistance to levofloxacin in *S. aureus* may result from mutations in topoisomerase genes and the presence of active efflux pumps (Zayed *et al*., 2015; Abd El-Baky *et al*., 2019). Cetirizine dihydrochloride was selected for its intrinsic antibacterial properties and known synergism with antibiotics El-Nakeeb *et al*., 2011). MIC values confirmed cetirizine activity at 512 µg/mL, while levofloxacin exhibited MICs of 32 µg/mL (*S. aureus*) and 16 µg/mL (*E. coli*) Areej *et al*., 2021). Broth microdilution results showed lower MICs for the combination: 8 µg/mL and 2 µg/mL, respectively. Checkerboard assays demonstrated synergism, with FIC indices of 1 µg/mL for *E. coli* and 0.5 µg/mL for *S. aureus* (Ahumada-Santos *et al*., 2016; Areej *et al*., 2021).

Genotoxicity of the combination was assessed using the comet assay, revealing minimal DNA damage at high concentrations (1024 µg/mL), with no damage at lower concentrations (Al-Soufi and Al-Rekabi, 2019). The combination showed a better safety profile compared to the control (20% DMSO). PCR and multiplex PCR confirmed the bacterial identity using 16S rRNA, nuc, and iss gene-specific primers (Ali *et al*., 2014; Azam *et al*., 2019).

While the study confirmed the synergistic effect of levofloxacin and cetirizine, limitations include its narrow focus on specific bacterial strains and lack of evaluation of other antibiotic combinations. Future studies should expand on these findings, exploring different clinical isolates and the molecular mechanisms underlying synergism.

Future implications

Antibiotic resistance is a growing global threat, making the discovery of new antibiotics to combat it

increasingly unviable. Consequently, we can leverage our obtained findings to introduce novel therapies for treating diverse infectious diseases by combining antibiotics with other drugs and chemicals. In the future, our study's efficient data will enhance the efficacy of antibiotics and prove valuable for increasing the effectiveness of drug treatments.

Conclusion

In summary, when administered alone, cetrizine demonstrates limited antibacterial activity against *E. coli* and *S. aureus*. However, when combined with levofloxacin, a synergistic effect is observed, resulting in a lower minimum inhibitory concentration (MIC) value. Furthermore, this combination therapy exhibits reduced toxicity compared to individual drug treatments. It is important to note that not only in vitro testing alone not achieve the modulation of antibiotic resistance. Therefore, it is recommended to conduct in vivo tests to further assess the antibacterial effectiveness of this drug combination.

Author contributions: ZA and UA conceptualized the study, designed the experiments, conducted data analysis, and wrote the first draft of the manuscript. AS AND ZA contributed to the study design, supervised the experimental work, and assisted in manuscript writing and revisions. MO performed data analysis, contributed to statistical interpretation, and reviewed the manuscript. MA assisted in conducting experiments, contributed reagents and materials, and participated in data collection. UA and IS contributed to manuscript writing, reviewed the final version, and provided critical feedback. All authors reviewed and approved the final manuscript.

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