The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 7571- 7575

Determination of Fractional Inhibitory Concentration (FIC) Index as A Measure of Synergy of Antibiotics in E. coli O157:H7

Sarah Sameer Abdulabbas[1], Zainab Khaleel Ibrahim[2], Hayder Ali Muhammed[3], Noor Ali Yazi AL-Khazali[4], Saja Talib Ahmed[5], Amir Fadhil Ai- Tuma[6] , Fatima Mohammed Saeed Mahdi[7]

[1,4,5,7] College of Pharmacy, University of Al-Zahraa for Women

[2] College of Medical and Health Techniques, University of Alkafeel, Iraq

[3] College of Veterinary Medicine, University of Kerbala. Iraq

[6] College of Medical and Health Technologies, University of Ahlalbayt,Iraq

Corresponding author: Zainab Khaleel Ibrahim**, E-mail:** Zainab.khalil@alkafeel.edu.iq **Mobile:** +9647715280528

ABSTRACT

Background: MIC is the lowest concentration of an antibiotic required to inhibit the growth of an organism.

Methods: MIC was done against six conventional antibiotics e.g., Amoxicillin, Ceftriaxone, Gentamycin, Tetracycline, Ciprofloxacin, and Levofloxacin. E. coli O157:H7 was collected from hospital. The antibacterial activity of six conventional antibiotics was assessed against E. coli O157:H7by using the broth microdilution method then the fractional inhibitory concentration (FIC) index was used to define the interactions between antibiotics.

Results: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antibiotics against E. coli O157:H7 showed that the highest concentration appeared in ciprofloxacin was 62.5 µg/ml. while ceftriaxone and gentamycin were 15.62 µg/ml. amoxicillin and levofloxacin was 7.812 µg/ml. the lowest concentration appeared in Tetracycline was 0.976 µg/ml. The higher FICI was seen in Ceftriaxone + Levofloxacin combination 0.3 % followed by Levofloxacin + Ciprofloxacin (0.1). **Conclusions:** The antibacterial activity of both Ceftriaxone and Levofloxacin was enhanced by the combination which proved a highly synergistic effect against E. coli O157:H7.

Keywords: MIC, MBC, FIC and E. coli O157:H7.

INTRODUCTION

One of the most serious illnesses is E. coli, the most prevalent commensal inhabitant of the gastrointestinal tracts of warm-blooded animals and humans. It is a member of the Enterobacteriaceae bacterial family [(1)].

Since the advent of various antibiotic resistance among members of the Enterobacteriaceae family is a major global public health concern, a significant fraction of serious, life-threatening infections in both community-acquired and nosocomial infections are caused by these bacteria [(2)]. While there are various ways that bacteria might resist antibiotics. The 'mobile' resistance genes found in the Enterobacteriaceae are responsible for the most significant resistance mechanism. Finding the gene(s) responsible for a specific phenotype can be difficult due to the enormous number of different genes that give resistance to each class or subclass of antibiotics [(3)].

The likelihood is that new medications will quickly lose their efficacy due to the rapid evolution of antibiotic resistance. Through the tailored combination of specific active agent qualities, researchers aim to be able to retain and enhance the efficiency of current antibiotics in the face of this dangerous circumstance. Combination therapy involves combining two or more medications to boost their effectiveness against bacteria that are resistant to common antibiotics [(4, 5)]. The minimum bactericidal concentration (MBC), which is the lowest concentration of antimicrobial agent needed to kill microorganisms, and the minimum inhibitory concentration (MIC), which is the lowest concentration of antimicrobial agent that prevents microbial growth, are the two main applications of the dilution method [(6)]. Since the early 1980s, the fractional inhibitory concentration (FIC) method has been applied to drugs, with a variety of operational approaches and conflicting result interpretations [(7, 10)]. The in vitro MICs and MBCs of various antibiotics against strain E coli O157:H7 were the focus of the current study's primary objective.

MATERIALS AND METHODS

Bacterial collection:

ATCC strain E. coli O157:H7 was collected from the Microbiology Laboratory [stock culture of E. coli O157:H7 where maintained in nutrient broth (HiMedia)] at Veterinary Medicine, Kerbala University, Iraq. E. coli O157:H7 was identified and confirmed phenotypically using theVitek-2 system (BioMerieux, Marcy L'Etoile, France).

Preparing of antibiotics stock solution:

To prepare a stock solution of Antibiotic agents, 55.35 g of Amoxicillin, 52.03 g of Ceftriaxone, 53.3 g of Gentamycin, 86.7 g of Tetracycline, 75.2 g of Ciprofloxacin, and 57.4 g of Levofloxacin dissolved in 10 mL distilled water each [(11)].

Determination of MIC: The antibacterial effectiveness of antibiotics was studied using the common broth dilution method (CLSI M07-A8) by observing the visible

Received: 17/07/2022
Accepted: 19/09/2022

growth of microorganisms in the agar broth. Six antibiotics chosen for the MIC and MBC in vitro investigation. One 96-well plate was used to test six antibiotics at a time, 1 ml of each antibiotic were transferred to the wells accordingly. MIC in BHI broth was calculated using serial two-fold dilutions of antibiotics at concentrations ranging from 500 mg/ml to 0.488 mg/ml with adjusted bacterial concentration (108 CFU/ml, 0.5 McFarland's standard). The positive control contained only E. coli O157:H7 and the negative control containing only antibiotic. Following a thorough antibiotics mix, E. coli O157:H7 was injected into each of the 10 dilutions. The infected microplate underwent a 24-hour overnight incubation at 37 °C. The antibiotics' minimum inhibitory concentration (MIC) against the studied bacteria E. coli O157:H7 was determined to be the antibiotics' greatest dilution to stop growth (with no turbidity in the tube) as shown in figure (1).

Determination of MBC: None of the plate's wells displayed any turbidity or apparent signs of growth (MIC and higher dilutions). Every sample was routinely cultured on MacConkey agar using the streak plate method in the lab (HiMedia). The agars were then incubated at 37°C for 24 hours overnight. The MBC value of the tested antibiotics against the tested bacterial E coli O157:H7 was determined as the lowest concentration at which no growth of the tested organisms was observed.

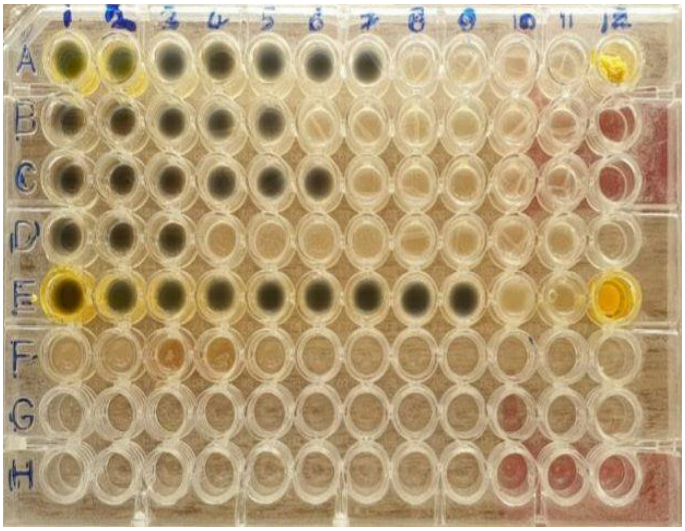

Figure (1): Microdilution checkerboard method for MIC.

Estimation of antibiotic combinations: The fractional inhibitory concentration index (FICI) for each double antibiotic combination was calculated using the concentrations in the first non-turbid well found in each row and column along the turbidity/non-turbidity interface and expressed as the median value [12]. The obtained FIC index then used in interpretation of interaction criteria of combination, which is illustrated in table (2) and figure (2). In order to interpret the outcomes of checkerboard experiments, the "fractional inhibitory concentration" (FIC) index (FICI) is computed as:

$$\frac{A}{MIC_A}+\frac{B}{MIC_B}=FIC_A+FIC_B=\textbf{FIC Index Value}$$

Ethical consideration:

The study was approved by The Local Medical Ethical Committee of the Veterinary Medicine College, Kerbala University, Iraq.

RESULTS

The MIC and MBC were calculated using the dilution method, and the results were obtained (which required inoculation onto agar plates devoid of antibiotic) as the antibiotic concentrations on a plate that, within the first 24 hours of incubation, caused bacterial growth or turbidity. The study made an effort to identify any synergism between antibiotics to treat challenging infections with medication resistance.

Following incubation in nutritional broth with various antibiotic concentrations at 15.62, 31.25, 125, 1.953, 31.25, and 15.62 µg/ml), as shown in table (1). According to table (1), the highest concentration appeared in ciprofloxacin was 62.5 µg/ml, while ceftriaxone and gentamycin were 15.62 µg/ml and for amoxicillin and levofloxacin was 7.812 µg/ml. The lowest concentration appeared in tetracycline was 0.976 µg/ml as shown in table (1) and figure (2). Inoculation of loopful of samples of the E. coli O157:H7 from wells before MIC (15.62, 31.25, 62.5, 3.90, 31.25, 15.62 µg/ml) from wells after MIC (3.906, 7.812, 31.25, 0.418, 7.812, 3.906 µg/ml) plate onto nutrient agar without antibiotic, and addition of equal volume of sterile nutrient broth without antibiotic (that is, double dilution)

In table (1), MBCs for E. coli O157:H7 were 15.62, 31.25, 62.5, 3.90, 31.25 and 15.62 µg/ml, using the DM, as there were no growth in E coli agar after 24 h incubation at 37°C. The study made an effort to identify any synergism between antibiotics to treat challenging infections with medication resistance. The checkerboard method of broth microdilution was used to compute MICs and to assess the interaction of six antibiotics in double combinations. As stated in the materials and methods, we computed the FICI, which is displayed in table (2), to find synergism. The FIC index, which was derived earlier and shown in table (2) and figure (3), was used to determine how different antibiotic combinations interacted with one another. When the FIC is greater than 4, the interaction type is deemed to have an antagonistic effect and when it is greater than 0.5, it has an additive effect; and when it is lower, it has a synergistic effect (FIC 1-4) [11].

Table (1): MIC and MBC of six antibiotics against E. coli O157:H7

NO.	Antibiotic	MIC (µg/ml)	MBC (µg/ml)
1	Amoxicillin	7.812	15.62
2	Gentamycin	15.62	31.25
3	Ciprofloxacin	62.5	62.5
4	Tetracycline	0.976	3.90
5	Ceftriaxone	19.62	62.5
6	Levofloxacin	7.912	15.62

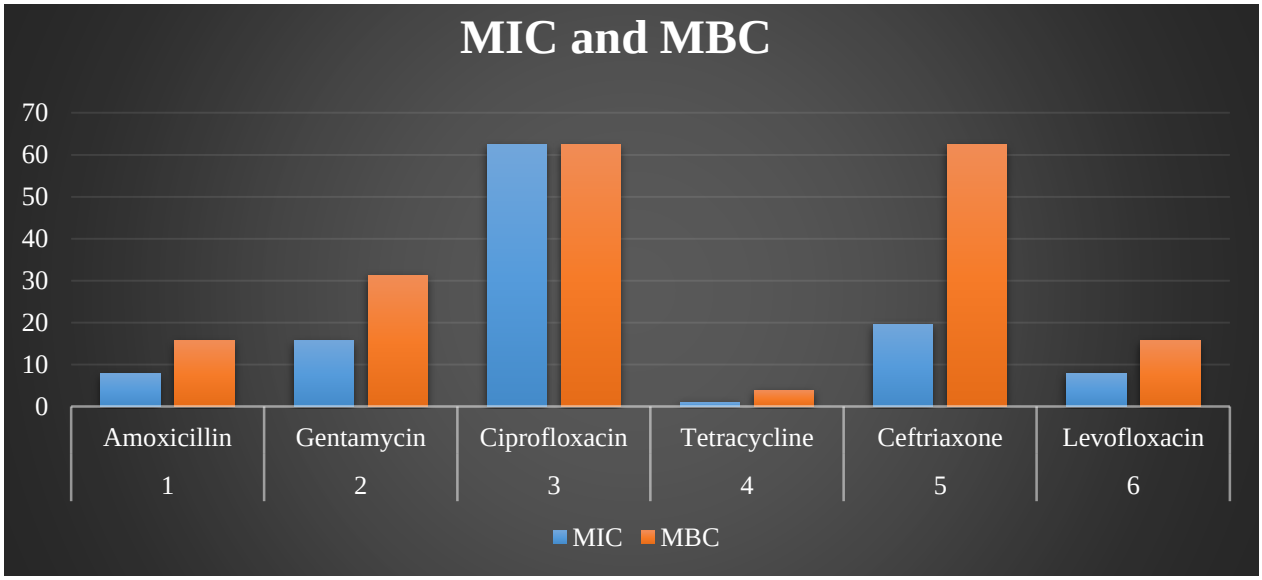

Figure (2): MIC and MBC of antibiotics towards Escherichia coli O157:H7.

Table (2): Summary of fractional inhibitory index outcome

NO.	Combination of antibiotic	FIC	Result
1	Amoxicillin +Tetracycline	4.5	Antagonism
2	Amoxicillin +Ceftriaxone	2.7	Indifferent
3	Amoxicillin + Levofloxacin	3.8	Indifferent
4	Amoxicillin + Ciprofloxacin	1.1	Indifferent
5	Gentamycin + Levofloxacin	1.5	Indifferent
6	Gentamycin + Ciprofloxacin	6.25	Antagonism
7	Gentamycin + Ceftriaxone	0.9	Additive
8	Ciprofloxacin + Ceftriaxone	4.1	Antagonism
9	Tetracycline + Ceftriaxone	1.04	Indifferent
10	Ceftriaxone + Levofloxacin	0.3	Synergism
11	Levofloxacin + Ciprofloxacin	0.1	Synergism

Antagonistic effect when (FIC of >4), or addition when (FIC > 0.5 <1), or synergistic effect when (FIC of ≤ 0.5), while indifference (FIC 1-4).

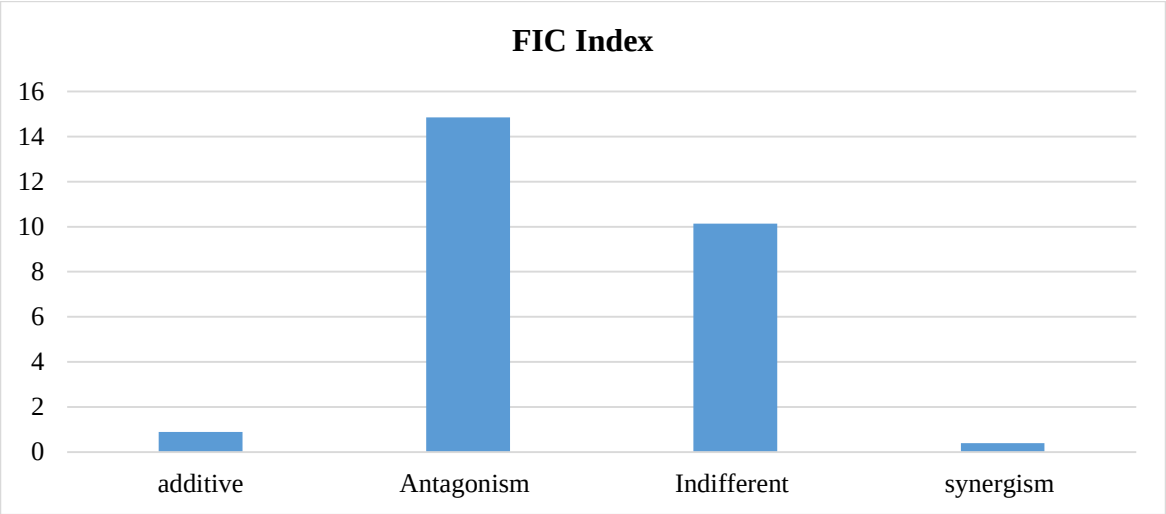

Figure (3): Combined effect of antibiotics expressed as fractional inhibitory concentration (FIC) index

DISCUSSION

Finding the MIC and MBC of antibiotics against E coli O157:H7 was the goal of this investigation. Agar diffusion and the MIC test are frequently used to evaluate a drug's antibacterial activity. In contrast to the agar diffusion method, direct contact tests have the advantage of being independent of the diffusion properties of the tested item and media [(13)]. To find the lowest concentration of a substance that will still exhibit antibacterial effects, a solution is serially diluted. Gram-negative bacteria linked to antibiotic resistance have become more prevalent over the past ten years, and these illnesses are now a serious danger to global public health [(14)]. They have a significant financial impact on healthcare systems due to increased expenditures brought on by prolonged hospital stays, in addition to being linked to unfavorable outcomes that worsen with time and more difficult treatments [(15)].

The hopeful researches of current antibiotics to better the confrontation with resistance to conventional treatment served as the driving force behind this study's attempt to uncover any synergism between antibiotics to treat challenging cases of resistant infections against single drug. The combination of Ceftriaxone + Levofloxacin was associated with a higher FICI of 0.3%, followed by Levofloxacin + Ciprofloxacin with 0.1%.

Antibiotics' synergistic effects were studied using the checkerboard microdilution technique. When the FIC is greater than 4, the interaction type is deemed to have an antagonistic effect; when it is greater than 0.5, it has an additive effect; and when it is lower, it has a synergistic effect (FIC 1-4). The first line of defense against microorganisms is combination therapy. A promising therapeutic approach involving two or more antibiotic combinations or antibiotics plus adjuvants is emerging [(16)].

Empirical antibiotic therapy is crucial in clinical settings since postponing effective antibiotic treatment can have negative therapeutic effects, especially in patients with severe illnesses. The choice of the proper empiric antibiotic therapy is influenced by a number of variables, but knowledge about the most likely bacterium may be the most important. The outcomes of the hospital microbiota's antimicrobial susceptibility profile can therefore be used as a crucial resource [(17)]. Levofloxacin, a fluoroquinolone antibiotic, and ceftriaxone, a cephalosporin, was generally the first pair of combinations to evaluate the synergism of double combinations.

Our findings support numerous earlier studies that found synergy between the two antibiotics. [(18,19, 20)]. The majority of E coli strains are part of the beneficial bacterial flora that lives in the human gut and are not toxic. However, some of them can make people sick. The most potent toxins that result in an intestinal infection are produced by some strains. Other strains of E coli infection can cause meningitis, pneumonia, respiratory diseases, and urinary tract infections [(21)].

By having hydrophilic channels called porins, Gram-negative bacteria control the properties of the outer membrane's permeability. Fluoroquinolones reach the porins and cross the membranes of the E. coli bacterial cell [(22).]

An antibiotic's ability to kill bacteria depends on both its capacity to enter the cell envelope and its effectiveness in attaching to the target location, in this instance DNA gyrase [(23).]

Three distinct pathways have been hypothesized for fluoroquinolones to enter the cell membrane of the Gram-negative organism: (i) The hydrophilic pathway through the porin channels [(20)], (ii) The membrane bilayer matrix's hydrophobic route [(22)], and (iii) The mechanism for self-promotional uptake [(26)]. Ceftriaxone, a third-generation

cephalosporin and beta-lactam antibiotic, has been shown to be effective against a wide variety of organisms and is characterized by relatively high stability towards the beta-lactamases of gram-negative bacteria [21].

CONCLUSIONS

Results of tests using Escherichia coli O157:H7 and the minimal inhibitory and bactericidal concentrations of the antibiotics Amoxicillin, Ceftriaxone, Gentamycin, Tetracycline, Ciprofloxacin, and Levofloxacin. The drugs' MIC and MBC against E. coli O157:H7 showed that ciprofloxacin had the highest concentration, which was 62.5 µg/ml. The study recommended Ceftriaxone and Levofloxacin as an efficient treatment for infections brought on by E coli O157:H7.

Funding: This work was funded by the corresponding author.

Authors Contribution: Sarah Sameer Abdulabbas, Zainab Khaleel Ibrahim, Hayder Ali Muhammed, Noor Ali Yazi AL-Khazali, Saja Talib Ahmed, Amir Fadhil Ai- tuma and Fatima Mohammed saeed Mahdi contributed equally.

Conflict of interest: The authors declared no conflict of interest.

REFERENCES

1. **Kaper J, Nataro J, Mobley H (2014):** Pathogenic Escherichia coli. Nat. Rev. Microbiol., 2: 123–140.
2. **Partridge S (2015):** Resistance mechanisms in Enterobacteriaceae. Pathology Journal, 47 (3): 276–284.
3. **Galal L, Abdel Aziz N, Hassan W (2018).** Defining the relationship between phenotypic and genotypic resistance profiles of multidrug-resistant enterobacterial clinical isolates. doi: 10.1007/5584_2018_208
4. **Worthington R, Melander C (2013).** Combination approaches to combat multidrug-resistant bacteria. *Trends in biotechnology*, *31*(3), 177-184.
5. **Boyd N, Nailor M (2011):** Combination Antibiotic Therapy for Empiric and Definitive Treatment of Gram-Negative Infections: Insights from the Society of Infectious Diseases Pharmacists. Pharmacotherapy, 31 (11): 1073–1084.
6. **Andrews J (2001):** Determination of minimum inhibitory concentrations. Journal of antimicrobial Chemotherapy, 48 (1): 5-16.
7. **Eucast F (2000):** Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. Clin. Microbiol. Infect., 6 (2): 503-508. 10.1046/j.1469-0691.2000.00149.x.
8. **Greco A, Greco G, Bravo J (1995):** The search for synergy: a critical review from a response surface perspective. Pharmacol. Rev., 47 (1): 331-385.
9. **Hall J, Hall F, Middleton D (1983):** The fractional inhibitory concentration (FIC) index as a measure of synergy. J. Antimicrob. Chemother., 11 (3): 427-433, 10.1093/jac/11.5.427.
10. **Odds F, Odds B (2003):** .Synergy, antagonism, and what the chequerboard puts between them. J. Antimicrob. Chemother., 52 (1): 12-24.
11. **Wayne P (2011):** Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 28th ed. Clinical and laboratory standards institute, 140-144.
12. **Tyers M, Wright G (2019):** Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nature Reviews Microbiology, 17 (3): 141-155.
13. **Estrela C, Rodrigues C, Bammann L *et al.* (2001):** Two methods to evaluate the antimicrobial action of calcium hydroxide paste. J Endod., 27 (12): 720–723.
14. **Ecdc S (2009):** The bacterial challenge: time to react, Ecdc/Emea. American Society for Microbiology Journals, 14 (7): 23-29.
15. **Vasoo S, Barreto J, Tosh P (2015):** Emerging Issues in Gram-Negative Bacterial Resistance: An Update for the Practicing Clinician. Mayo Clinic Proceedings, 90 (3): 395– 403.
16. **Cottarel G, Wierzbowski J (2007):** Combination drugs, an emerging option for antibacterial therapy. Trends in Biotechnology, 25 (12): 547–555.
17. **Leekha, S., Terrell, C. L., & Edson, R. S. (2011, February).** General principles of antimicrobial therapy. In Mayo clinic proceedings, 86 (2): 156-167.
18. **Glew R, Pavuk R (1983):** Early synergistic interaction between semisynthetic penicillins and aminoglycosidic aminocyclitols against Enterobacteriaceae. American Society for Microbiology Journals, 23 (6): 902–906.
19. **Al-Muharrmi Z (2008):** Antibiotic combination as empirical therapy for extended spectrum beta-lactamase, Oman Medical Journal, 23 (2): 78–81.
20. **Lim C, Spelman D (2019):** Mortality impact of empirical antimicrobial therapy in ESBL- and AmpC-producing Enterobacteriaceae bacteremia in an Australian tertiary hospital. Infection, Disease and Health Journal, 24 (3): 124–133.
21. **Aburawi S, Doro B, Awad E (2019):** Effect of ciprofloxacin on S. aureus and E. coli growth in presence of vitamin C using cup cut diffusion method. Journal of Pharmacy and Pharmacology, 7 (9): 473-484.
22. **Nikaido H, Vaara M (1985):** Outer Membrane Permeability and Antibiotic Resistance. Microbiol. Rev., 49 (7): 1-32.
23. **Maina D, Revathi G, Kariuki S, Ozwara H (2012):** Genotypes and cephalosporin susceptibility in extended-spectrum beta-lactamase producing enterobacteriaceae in the community. Journal of Infection in Developing Countries, 6 (6): 470–477. https://doi. org/10.3855/jidc.1456.