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ORIGINAL ARTICLE

Reduce antibacterial activity against ceftriaxone resist Escherichia coli isolated from urinary tract infections by modification of ceftriaxone

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Abstract

This study focused on clarifying the problem of antibiotic resistance in harmful bacteria and proposed a solution by utilizing the advantages of schiff base synthesis. Seventy-five urine samples were procured from a Baghdad, Iraq medical facility, forming the study's basis. Through a chemical reaction involving ceftriaxone and specific aldehydes, namely 4-hydroxybenzaldehyde and 4-ethoxybenzaldehyde, two novel Schiff base derivatives, labeled as compounds A and B, were synthesized and subsequently characterized using Fourier-transform infrared spectroscopy (FTIR). Intriguingly, conventional aldehyde derivatives and ceftriaxone exhibited limited efficacy in inhibiting Escherichia coli (E. coli) growth, as evidenced by reduced inhibition zones. In contrast, the newly synthesized Schiff base derivatives demonstrated enhanced antimicrobial activity, significantly inhibiting bacterial growth with zones measuring 21 mm for compound A and 18 mm for compound B. These findings underscore the potential of Schiff base synthesis as a promising avenue for developing effective strategies against antibiotic-resistant bacterial strains, highlighting the importance of innovative approaches in combating microbial resistance.

1. Introduction

Antimicrobial resistance, primarily caused by bacteria, is a prominent global public health problem [1]. The increasing antibiotic resistance in bacteria that are significant human pathogens, along with the transmission of resistance from the confined setting of hospitals to wider communities, poses a severe threat to public health [2]. Ceftriaxone is anticipated to exhibit efficacy against many gram-positive and gram-negative microorganisms [3].

Developing countries encounter unique challenges, from self-prescribing antibiotics to inadequate sanitary conditions, including those found in hospitals [4]. These conditions contribute to the emergence and spread of specific multi-resistant pathogens uncommon in developed countries. Furthermore, more research is needed into new antibiotics to combat these particular pathogens [5]. Moreover, it is crucial to acknowledge that these idiosyncrasies can readily transcend national boundaries in the current era of globalization, thereby impacting resistance patterns worldwide [6]. Different microorganisms like bacteria, fungi, and actinomycetes produce chemical compounds known as antibiotics [7], also known as antibacterial or chemotherapeutic medications [8]. These compounds can impede or eradicate other microorganisms. Antibiotics are chemically created medicines

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that are not biologically derived, despite being called "antibiotics." A more appropriate designation is antibacterial/chemotherapeutic agents [9]. Ceftriaxone, a cephalosporin of the third-generation antibiotic, is commonly employed for the treatment of invasive infections caused by Enterobacteriaceae [10], including Escherichia coli. The rising global prevalence of antimicrobial resistance (AMR) in Enterobacteriaceae is leading to higher rates of patient illness and death [11], increased expenses in healthcare, and more reliance on medicines that are used as a last resort. The World Health Organization has acknowledged ceftriaxone-resistant Enterobacteriaceae as a crucial priority pathogen. Extended-spectrum ß-lactamases (ESBLs) and AmpC ß-lactamases are the primary mechanisms of ceftriaxone resistance in Enterobacteriaceae [12]. The genes responsible for generating these enzymes can be horizontally transmitted from one bacterium to another, which facilitates the dissemination of resistance [13].

This study aimed to determine how well ceftriaxone and schiff bases kill *E. coli* strains resistant to Ceftriaxone.

2. Methods

2.1 Synthesis of Schiff base derivatives (A and B)

For ceftriaxone modification as Schiff base derivatives, dissolve 4-hydroxybenzaldehyde (1.22 gm, 0.01 mol) and 4-ethoxybenzaldehyde (1.5 gm, 0.0 mol), respectively, in 20 ml of absolute ethanol. Then, the ceftriaxone (5.54 gm, 0.01 mol) was added to these solutions and refluxed for 3 h at 70 °C with stirring. After 4 hours, the solutions were cooled to room temperature. The yellow precipitates were filtered, washed with ethanol, and dried in a vacuum [14]. The structure of these derivatives is shown in Fig .1.

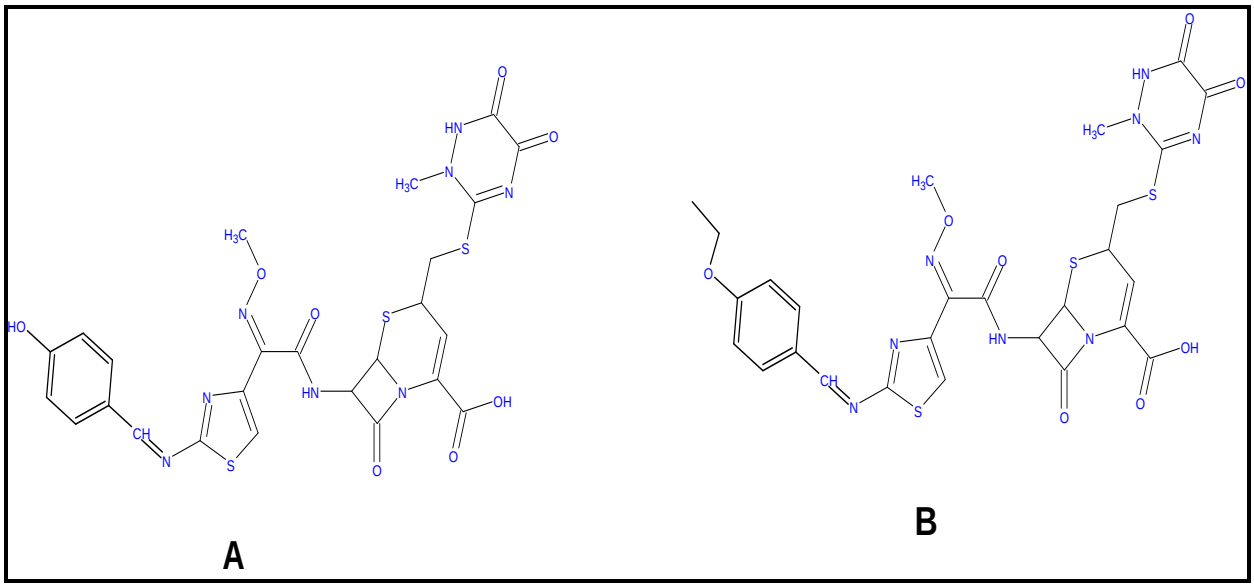

Figure 1: Structures of Schiff base (A and B).

2.2 Antimicrobial Activity of Schiff base derivatives (A and B)

The *E. coli* bacteria were cultivated on Muller-Hinton dishes via a sterile loop and streaking techniques, starting with the broth [15]. Subsequently, a single well was generated in the agar. Each well received 100 µl of the relevant dilution of Schiff base derivatives A and B (ceftriaxone and aldehyde derivatives). The dish was hermetically sealed and incubated at 37 ºC for the night to be examined the following day.

2.3 Dilution of Ceftriaxone

With a micropipette, 100 µl of the stock solution (made by dissolving 1 gm of the antibiotic in 10 ml of distilled water) was added to the distilled water (900 µl) in an Eppendorf tube to produce a solution (1). Three dilutions were prepared using the serial dilution approach [16].

2.4 Resistance to Ceftriaxone

E. coli bacteria were cultured in a media (Muller-Hinton), and a ceftriaxone disk (40 µg) was placed onto the agar. The bacteria grew exponentially overnight [17].

2.5 Experimental of Docking

The protein data bank (PDB code: 1MOQ) was used to get the crystallographic 3D structure of glucose-6-phosphate synthase (GP6 synthase). The enzyme structure was put through energy minimization using Swiss Protein Viewer (version 4.1). Several preparatory stages were executed, resulting in the removal of molecules of water and co-crystallized compounds. Subsequently, the structure was repaired, and polar hydrogens were included. The compounds produced were shown using ChemDraw Ultra (version 18.0) and stored in a sdf format file.

The energy minimization was performed via Chem3D (version 18.0), then converted to a pdb file format by OpenBabel software. We used Autodock Tools (version 1.5.6) [18, 19], Eberhardt, Santos-Martins et al. 2021) to do the docking process. We saved the results in pdbqt file format after minimizing the energies of both enzymes and ligands. The molecular docking was conducted using Autodock Vina with the AutoGrid program. The grid box's dimensions were 40 x 40 x 40, with a grid center of 21.067 x 20.007 x 12.729, representing the x, y, and z dimensions, respectively. The docking settings were configured with the default values, resulting in the generation of nine conformations. Discovery Studio was used to generate display pictures [20].

3. Results and Discussion

FTIR (cm^{-1}) of Schiff base derivative (A): the broad band of hydroxyl group appeared at 3359, C-H aromatic at 3089, C-H aliphatic appeared at 2934 and 2882, and the carbonyl group of carboxylic acid and amide appeared at 1718 and 1668, respectively. The amine group of Schiff base at 1642 and C-C aromatic at 1592 [21], as shown in Fig. 2.

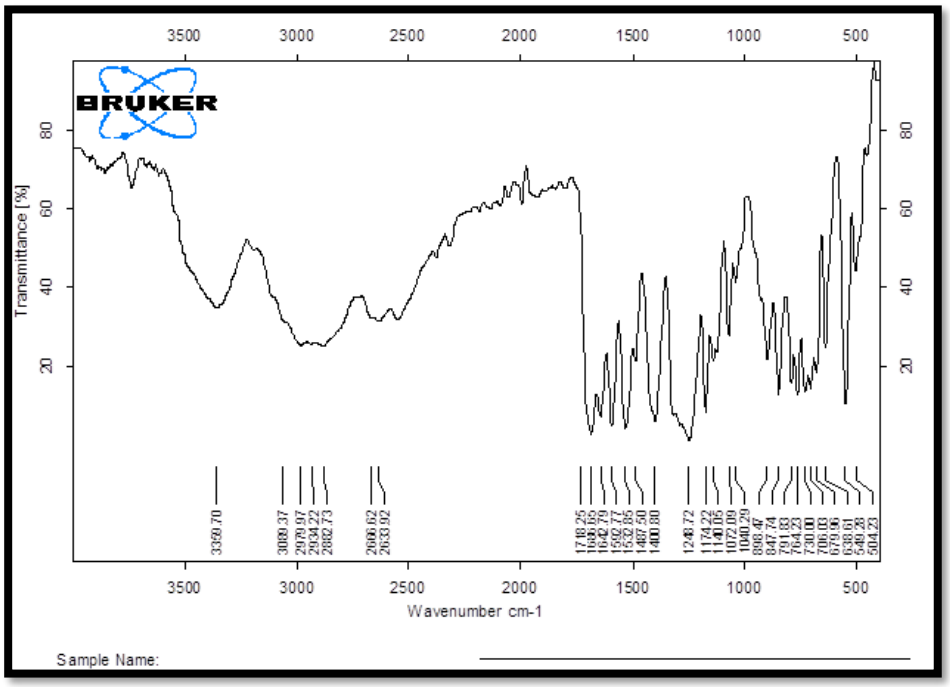

Figure 2: FTIR for derivative A.

FTIR (cm^{-1}) of schiff base derivative (B): the broad band of hydroxyl group appeared at 3340, C-H aromatic at 3046, C-H aliphatic appeared at 2940 and 2880, and the carbonyl group of carboxylic acid and amide appeared at 1717 and 1690, respectively. The imine group of Schiff base at 1642 and C-C aromatic at 1593 [22], as shown in Fig. 3.

3.1 Discussion of Docking

Docking models are crucial for investigating the binding mechanisms of ligands to a target molecule. The significance of glucosamine-6-phosphate synthase (GP6 synthase) in microbial cell wall formation has garnered attention from numerous researchers [23]. In the first step of hexosamine biosynthesis, the enzyme speeds up the process by changing fructose-6-phosphate into glucosamine-6-phosphate. GlcN-6-P is a building block for uridine diphosphate N-acetyl glucosamine (UDP-NAG), which is an important part of the peptidoglycan layer in the cell walls of microbes [24]. The docking profiles of ceftriaxone and its derivative are shown in Fig. 4–7. The 3D conformations of both ligands show multiple interactions, most notably hydrogen bonding, in addition to other interactions such as van deer Waals and pi-alkyl. The binding affinities for both ligands were comparable, with computed values of -6.9 kcal/mol and -7.3 kcal/mol for ceftriaxone and ethoxy-ceftriaxone, respectively. Experimental findings further support the findings that both ligands exhibit anti-bacterial activity.

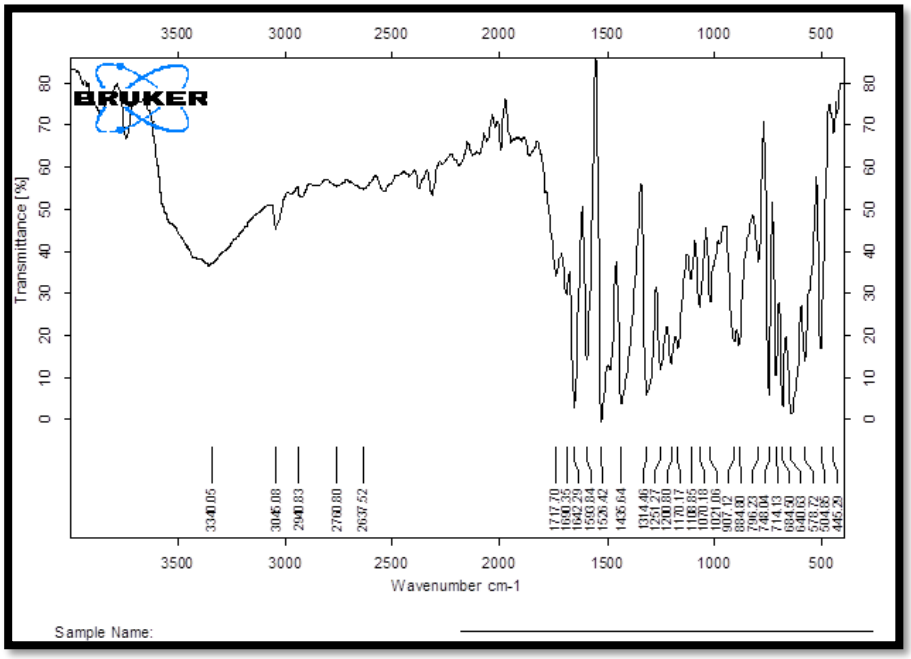

Figure 3: FTIR for derivative B

3.2 Aldehyde Derivatives (4-hydroxybenzaldehyde and 4-ethoxybenzaldehyde) Sensitivity Test

0.05 µg/µl Schiff base derivatives (A and B) were added to wells on Muller-Hinton agar. Subsequently, the plates were infected with an overnight culture of bacteria. The next day, the data and the antibacterial activity of Schiff base derivatives (A and B) against *E. coli* bacteria were looked at. The results showed 6 and 5 mm of inhibition zones, respectively. The Dens Check machine was employed to standardize the first Kahn tube to the McFarland standard (1.5 x 108 CFU/ml) [25].

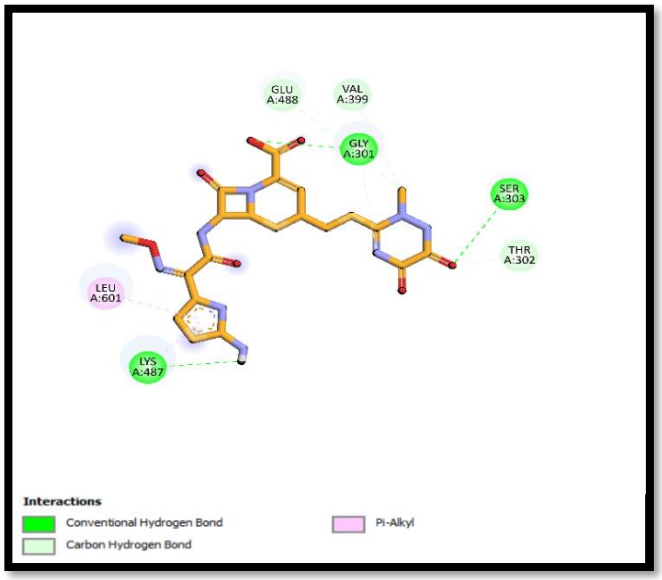

Figure 4: Docking profile 2D of ceftriaxone against GP6 synthase.

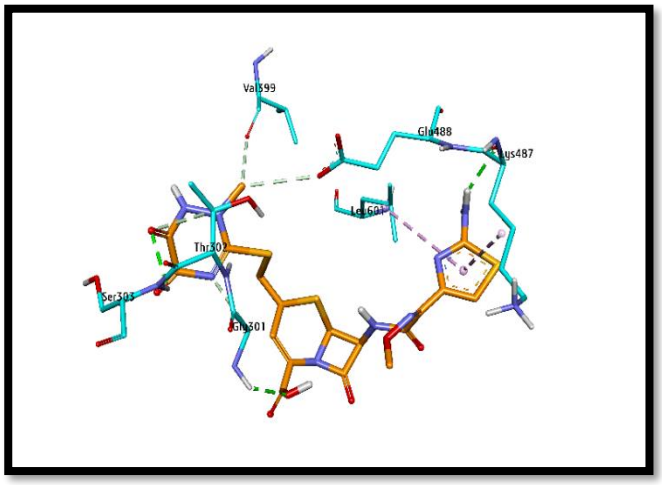

Figure 5: Docking profile 3D of ceftriaxone against GP6 synthase.

3.3 Sensitivity Test of Ceftriaxone

E. coli was cultured overnight on Muller-Hinton agar in the presence of a ceftriaxone disk (40 µg). Upon analysis overnight, the results showed 10 mm of inhibition zones of the growth zone [26].

3.4 Ceftriaxone minimum inhibitory concentration test

Ceftriaxone, used at a dose of 80 µg/µl, clearly inhibits the investigated isolates. The diameter of the inhibition zone in comparison to the antibiotic disc determines the degree of inhibition. However, no antibacterial effect was observed when the antibiotic was tested at different concentrations of 10, 5, and 1 µg/µl, demonstrating the dilutions' effect on *E. coli*.

The antibacterial properties of the Schiff base exhibited significant inhibitory activity, resulting in inhibition zones measuring 21 and 18 mm, respectively. In comparison, 4-hydroxybenzaldehyde and 4-ethoxybenzaldehyde, when applied individually, only produced inhibition zones measuring 6 and 5 mm, respectively. Fig. 8 displays the inhibition areas observed due to combining ceftriaxone with Schiff base (A) [27].

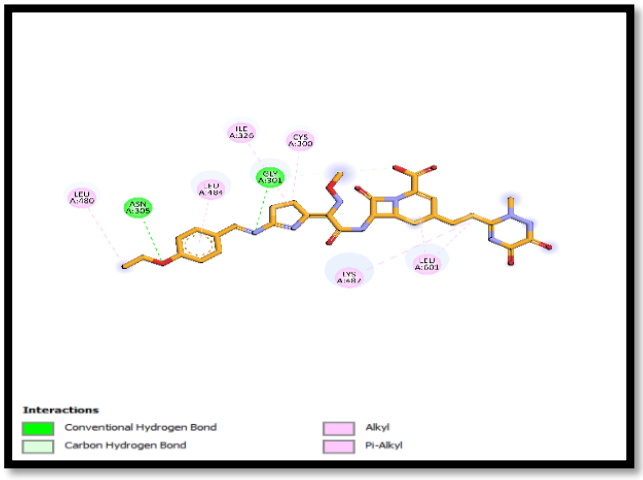

Figure 6: Docking profile 2D of Schiff base derivative (B) against GP6 synthase.

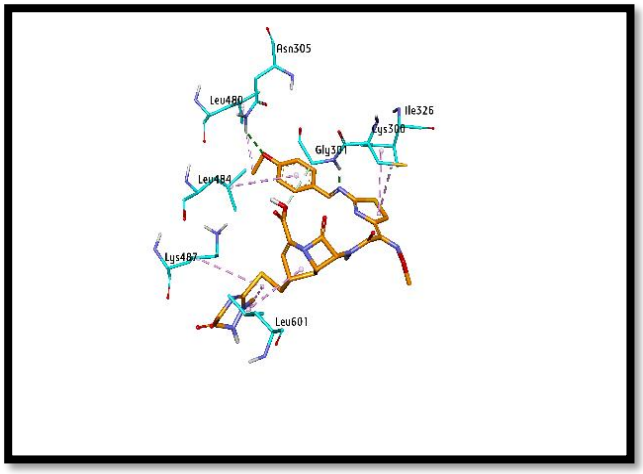

Figure 7: Docking profile 3D of Schiff base derivative (B) against GP6 synthase.

Based on the results obtained, the Schiff base derivatives of Ceftriaxone demonstrate a higher capacity to hinder the development of *E. coli* on Muller-Hinton agar. As previously stated, the absence of any inhibitory zone indicates that *E. coli* exhibited resistance to the presence of unbound Ceftriaxone. The current study also shows that free aldehyde derivatives can prevent *E. coli* from growing near the aldehyde derivatives well, creating a 6- and 5-mm wide inhibition zone [28].

The combination of aldehyde derivatives with Ceftriaxone has resulted in enhanced bactericidal activity of the Schiff bases (ceftriaxone and aldehyde derivatives), with killing zones of 21 and 18 mm for the initial dosage of aldehyde derivatives and the first dilution of Ceftriaxone, respectively. Table 1 illustrates the different concentrations of Schiff bases (A and B) along with their corresponding killing zone diameter and the percentage increase for each concentration.

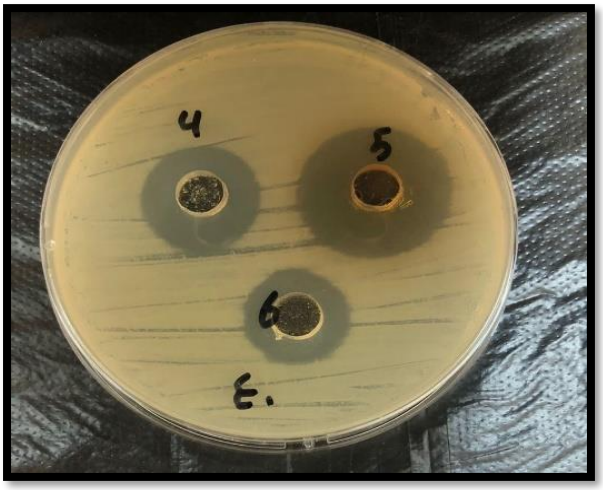

Figure 8: Schiff base derivative (A) on Muller–Hinton agar.

Studies have demonstrated that Schiff base derivatives of ceftriaxone modification identified with antibiotics may increase the levels of antibiotics at the location where bacteria and antibiotics come into contact and facilitate the binding of antibiotics to microorganisms.

Juan A. et al., Synthesized a tridentate schiff base derived from cephalexin. The present finding confirms that ceftriaxone effectively produces synergistic antibacterial actions against several types of bacteria. In addition, cephalexin, along with other cephalosporins, has shown increased antibacterial efficacy against bacteria when combined with aldehyde derivatives [29], which agrees with our study.

Abdelbaset A., Schiff bases are derived from cephalexin. In these derivatives, an amino group available in the drug substances was allowed to react with two derivatives of aldehyde separately to obtain Schiff bases [30]. This study agreed with ours.

Table 1: Inhibition zone of E. coli.

No. of comparison	4-hydroxybenzaldehyde	4-ethoxybenzaldehyde	Schiff base (A)	Schiff base (B)
Comparison #1	6	5	21	18
Comparison #2			20	16
Comparison #3			18	15

4. Conclusions

In this study, we successfully synthesized two novel Schiff base derivatives, namely compounds A and B, through the chemical reaction involving ceftriaxone and specific aldehydes: 4-hydroxybenzaldehyde and 4-ethoxybenzaldehyde. To validate their molecular composition and structure, these derivatives underwent characterization using Fourier-transform infrared spectroscopy (FTIR). Intriguingly, when these Schiff base derivatives were combined with ceftriaxone, they manifested a synergistic effect. This synergism notably amplified the inhibition zone, indicating enhanced efficacy against the targeted agents or organisms. Despite these promising observations, a comprehensive understanding of this synergistic interaction's underlying mechanisms and full potential still needs to be discovered. Therefore, further intricate studies and analyses are imperative to elucidate the precise nature of this synergistic impact. Such in-depth investigations will validate our initial findings and pave the way for potential applications and advancements in effectively combating relevant challenges.

References

[1] Mancuso, G., et al. (2021). "Bacterial antibiotic resistance: The most critical pathogens." Pathogens **10**(10): 1310.

[2] Ferri, M., et al. (2017). "Antimicrobial resistance: A global emerging threat to public health systems." Critical reviews in food science and nutrition **57**(13): 2857-2876.

[3] Khan, M. Y., et al. (2017). "A review-ceftriaxone for life." Asian Journal of Pharmaceutical Research **7**(1): 35-48.

[4] Belkina, T. (2017). "Non-prescribed antibiotic use in some developing countries and its association with drug resistance."

[5] Sakeena, M., et al. (2018). "Non-prescription sales of antimicrobial agents at community pharmacies in developing countries: a systematic review." International journal of antimicrobial agents **52**(6): 771-782.

[6] Fekadu, G., et al. (2020). "Self-medication practices and associated factors among health-care professionals in selected hospitals of Western Ethiopia." Patient preference and adherence: 353-361.

[7] Hutchings, M. I., et al. (2019). "Antibiotics: past, present and future." Current opinion in microbiology **51**: 72-80.

[8] Mohr, K. I. (2016). "History of antibiotics research." How to Overcome the Antibiotic Crisis: Facts, Challenges, Technologies and Future Perspectives: 237-272.

[9] Butler, M. S. and D. L. Paterson (2020). "Antibiotics in the clinical pipeline in October 2019." The Journal of antibiotics **73**(6): 329-364.

[10] Zeng, L., et al. (2020). "Safety of ceftriaxone in paediatrics: a systematic review." Archives of disease in childhood.

[11] Tewabe, A., et al. (2021). "The contribution of nano-based strategies in overcoming ceftriaxone resistance: a literature review." Pharmacology Research & Perspectives **9**(4): e00849.

[12] De Angelis, G., et al. (2020). "Molecular mechanisms, epidemiology, and clinical importance of β-lactam resistance in Enterobacteriaceae." International journal of molecular sciences **21**(14): 5090.

[13] Sharma, M. K., et al. (2022). "Treatment of MDR Gram-Negative Bacteria Infections: Ongoing and Prospective." Journal of Pure & Applied Microbiology **16**(3).

[14] Tokalı, F. S., et al. (2021). "Synthesis, characterization, biological activity and molecular docking studies of novel schiff bases derived from thiosemicarbazide: Biochemical and computational approach." Journal of molecular Structure **1231**: 129666.

[15] Al Kafaween, M. A., et al. (2019). "Effect of Trigona honey on Escherichia coli cell culture growth: In vitro study." Journal of Apitherapy **5**(2): 10-17.

[16] Kabwe, M., et al. (2021). "Isolation and Functional Characterization of Fusobacterium nucleatum Bacteriophage." The Oral Microbiome: Methods and Protocols: 51-68.

[17] Chua, K. Y. and A. J. Stewardson (2019). "Individual and community predictors of urinary ceftriaxone-resistant Escherichia coli isolates, Victoria, Australia." Antimicrobial Resistance & Infection Control **8**(1): 1-11.

[18] Trott, O. and A. J. Olson (2010). "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading." Journal of computational chemistry **31**(2): 455-461.

[19] Eberhardt, J., et al. (2021). "AutoDock Vina 1.2. 0: New docking methods, expanded force field, and python bindings." Journal of chemical information and modeling **61**(8): 3891-3898.

[20] Nielsen, T. R., et al. (2012). "Antimicrobial activity of selected South African medicinal plants." BMC complementary and alternative medicine **12**: 1-6.

[21] El-Sonbati, A., et al. (2019). "Synthesis, characterization of Schiff base metal complexes and their biological investigation." Applied Organometallic Chemistry **33**(9): e5048.

[22] Yousif, E., et al. (2017). "Metal complexes of Schiff base: preparation, characterization and antibacterial activity." Arabian Journal of Chemistry **10**: S1639-S1644.

[23] Lather, A., et al. (2021). "Aesculin based glucosamine-6-phosphate synthase inhibitors as novel preservatives for food and pharmaceutical products: in-silico studies, antioxidant, antimicrobial and preservative efficacy evaluation." BMC chemistry **15**(1): 1-11.

[24] Lather, A., et al. (2020). "Naringin derivatives as glucosamine-6-phosphate synthase inhibitors based preservatives and their biological evaluation." Scientific Reports **10**(1): 20477.

[25] Stompor, M. and B. Żarowska (2016). "Antimicrobial activity of xanthohumol and its selected structural analogues." Molecules **21**(5): 608.

[26] Masood, S. H. and N. Aslam (2010). "In vitro susceptibility test of different clinical isolates against ceftriaxone." Oman medical journal **25**(3): 199.

[27] Aunkor, M. T. H., et al. (2020). "Antibacterial activity of graphene oxide nanosheet against multidrug resistant superbugs isolated from infected patients." Royal Society open science **7**(7): 200640.

[28] El-Samanody, E. S. A., et al. (2018). "Molecular modeling, spectral investigation and thermal studies of the new asymmetric Schiff base ligand;(E)-N'-(1-(4-((E)-2-hydroxybenzylideneamino) phenyl) ethylidene) morpholine-4-carbothiohydrazide and its metal complexes: Evaluation of their antibacterial and anti-molluscicidal activity." Applied Organometallic Chemistry **32**(4): e4262.

[29] Anacona, J., et al. (2016). "Synthesis, characterization and antibacterial activity of a tridentate Schiff base derived from cephalexin and 1, 6-hexanediamine and its transition metal complexes." Med chem **6**: 467.

[30] Elgellal, A. A. and A. M. Alshadly (2014). "Preparation characterization and biological evaluation of Schiff-Base of some drug substances." Journal of Medical and Bioengineering Vol **3**(3).

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