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Test the effectiveness of some medications and vitamin D3 used against COVID-19 on Gram-positive and Gram-negative bacteria isolated from people infected with the Coronavirus

Amal F. AL-Gorani [1a], Hanan B. Saadon [2b*] , Ahmed. K. Ajeel [3c] , Noor Abd alkhudhur Salman[4d], Aya Talib Jawad[2e], Hussein Adnan Obaid[2f] and Hasan Mohsen Koti[5g]

[1] *Department of Biology, College of Science, University of Thi-Qar, Iraq.*
[2] *Department of Path. Analysis, College of Science, University of Thi-Qar, Iraq.*
[3] *Ministry of Education, General of Directorate of Edu, Thi-Qar, Iraq.*
[4]*Department of Chemistry, College of Science, University of Thi-Qar, Iraq.*
[5]*Department of Animal Physiology, Science and research Branch, Islamic Azad University, Iran.*
[a]E-mail: amal.fal.bio@sci.utq.edu.iq , [c]E-mail: Ahmed.ajeel88@utq.edu.iq
[d]E-mail: noor.abdulkhudur@sci.utq.edu.iq , [e]E-mail: Aya.talib@sci.utq.edu.iq
[f]E-mail: Hussain.adnan@sci.utq.edu.iq , [g]E-mail: Hassanalgrabat@gmail.com
[b*]Corresponding author: hananaljaberi@sci.utq.edu.iq

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***Abstract*— The COVID-19 pandemic has led to a search for effective pharmaceuticals and vitamins to combat the virus. These therapies have antiviral capabilities and can affect both gram-negative and gram-positive bacteria. This study aims to assess the effectiveness of various medications and nutrients in treating bacterial infections linked to COVID-19. In vitro, tests will be conducted to evaluate these therapies' antibacterial efficacy against both gram-negative and gram-positive bacteria.**

In the current study, we test a group of antibiotics commonly used in treating Coronavirus on different types of Gram-positive and Gram-negative bacteria. They found that Azithromycin was highly effective against both positive and negative bacteria, but its effectiveness decreased when combined with vitamin D. Levofloxacin was the most effective antibiotic, and its efficacy was not affected by the addition of zinc or vitamin D. Acyclovir had a slight effect on bacteria when used alone, but its efficacy improved with zinc. Zinc and Vitamin D had a high degree of effectiveness, but Vitamin D had relatively low efficacy, except against one type of bacteria.

The study aims to evaluate the effectiveness of drugs and vitamins against bacterial infections in COVID-19 patients. The results will provide a better understanding of the potential advantages of these medications in controlling bacterial infections. (The abstract should have one paragraph)

Keywords— COVID-19, Antibiotics, Antimicrobial activity.

I. INTRODUCTION

Coronaviruses, a subfamily within the Nidovirales order, are frequently encountered pathogens in humans. These viruses have an enveloped structure and contain single-stranded RNA with a positive sense. They are classified under the Coronavirdiae family and have been linked to a range of illnesses in both humans and animals, including acute respiratory, hepatic, and neurological diseases of differing degrees of severity [1-3]. Coronaviruses are categorized into four genera: alpha coronavirus (αCoV), beta coronavirus (β CoV), gamma coronavirus (γ CoV), and delta coronavirus (δ CoV). Notably, two novel β CoVs, namely SARS-CoV and MERS-CoV, have been identified with high mortality rates. Evolutionary studies indicate that αCoVs and βCoVs are predominantly present in bats and rodents, while δ CoVs and γ CoVs are primarily found in birds. These viruses have demonstrated the ability to cross species barriers frequently and pose significant threats as human pathogens [1], [4].

On December 31, 2019, cases of pneumonia with an unknown cause emerged in Wuhan city, Hubei Province of China. Chinese authorities identified the causative agent as a novel coronavirus and named the disease COVID-19, short for coronavirus disease(COVID-19). The disease's incubation period ranges from 2 to 14 days, with 80% of patients experiencing mild or asymptomatic illness, while 15% and 5% exhibit severe and critical cases, respectively. The etiology of COVID-19 is known as SARS-CoV-2 and belongs to the beta coronavirus group, as reported by the International Committee on Taxonomy of Viruses (ICTV) [5,6]. The COVID-19 pandemic has prompted the implementation of many vaccine initiatives and pharmaceutical treatment protocols. Presently, researchers are directing their attention toward bacterial infections in individuals with COVID-19, where severe viral pneumonia is identified as the primary factor contributing to mortality [7,8]. Corticosteroids have demonstrated efficacy in reducing mortality in cases of COVID-19 pneumonia. However, the use of immunosuppressive therapy and hospitalization increases the risk of bacterial infections. During the initial surge of the pandemic, 8.5% of hospitalized patients experienced bacterial infections [9]. However, it is common for individuals with COVID-19 to be administered drugs in

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regular medical practice, which could potentially result in a rise in drug-resistant bacteria [3], [10,11]. Antimicrobials have multiple potential functions in the management of COVID-19. Research is currently being conducted to investigate potential remedies for the treatment of SARS-CoV-2 [6]. Available data indicates that nosocomial infections are linked to an increased likelihood of mortality and severity of COVID-19 [7], [12]. Additionally, the presence of antibiotic resistance among the microorganisms responsible for subsequent infections poses a concealed danger that exists within COVID-19 patients. A significant number of hospital-acquired infections (HAIs), including hospital/ventilator-associated pneumonia, bloodstream infection, and urinary tract infection (UTI), may remain unreported due to the absence of culture protocols or inadequate culturing facilities in several hospitals [13].

Vitamin D is crucial for optimal bone strength, although the majority of Americans have insufficient amounts for optimal bone health. Individuals with insufficient vitamin D levels are also at an elevated risk of developing obesity, hypertension, depressive symptoms, sleep disturbances, as well as certain infections such as COVID-19 and influenza [14-16]. For optimal bone health, the majority of individuals should consume a daily dosage of 600-800 international units (IU) of vitamin D. Nevertheless, it remains uncertain if the recommended doses for bone health can prevent COVID-19and influenza [10]. Azithromycin Zinc combination provides antiviral, anti-inflammatory, and immunomodulatory advantages, making it a promising treatment for flu-like COVID-19 and atypical pneumonia. Moreover, the bactericidal effect of AZM may interfere with the symbiotic relationship between bacteria and viruses, a phenomenon that lacks sufficient documentation [17]. Acyclovir, as the primary antiviral treatment, this medication has proven to be effective, safe, and affordable in mitigating the immediate and potential long-term effects of SARS-CoV-2 virus infection in individuals. It has been shown to be beneficial in preventing hospitalization in high-risk individuals with mild to severe illness [18,19]. Levofloxacin, a fluoroquinolone antibiotic, is being explored for repurposing as an additional treatment for COVID-19. The Annals of Clinical Microbiology and Antimicrobials released a study that examined the use of levofloxacin and other antibiotics for treating COVID-19 [20, 21]. The objective of this study is to demonstrate the influence of specific therapies administered to persons infected with the Coronavirus (COVID-19) on the bacterial species isolated from them.

II. MATERIALS AND METHODS

A. Collection of samples

The study included 65 samples taken in the form of swabs samples from patients suffering from COVID-19 infections who attended AL-Hussein Teaching Hospital in Nasiriya city south of Iraq and their different ages of both genders, for the period from October 2023 to the end of December 2023. The information for the research was recorded in a questionnaire form for information about the person within the subject of the study, the swabs were put into a tube containing amines transport media and then sent to the laboratory. Collecting bacterial samples from COVID-19 infected individuals requires proper handling and safety precautions. The standardized clinical procedure for collecting bacterial samples from individuals with bacterial infections is usually done through endotracheal aspirate (ETA) specimens collected in sterile tubes. For this study, special types of Gram-negative (Escherichia coli, Klebsiella pneumonia) and Gram-positive bacteria (Staphylococcus aureus, Streptococcus) were collected. The objective of the study is to analyze the impact of different antimicrobial agents and vitamins (mentioned in Table (1)) on bacteria. The substances are categorized into three groups:

Group (1) consisted of Azithromycin (A), Acyclovir (B), Levofloxacin (C), Zinc (Z), and Vitamin D3.

Group (2) included Azithromycin + Zinc (A1), Acyclovir + Zinc (B1), and Levofloxacin + Zinc (C1).

Lastly, Group (3) comprised of Azithromycin +VitaminD3 (A2), Acyclovir + Vitamin D3 (B2), and Levofloxacin +VitaminD3 (C2).

Table (1): Drugs.

No	Drugs	Origin
1	Azithromycin (500 mg)	Stanford Biotech Ltd (India)
	Levofloxacin (500 mg)	Sapiens pharmaceutical Ltd (Cyprus)
	Acyclovir (400 mg)	Medyplex Pharmaceutical Ltd (India)
	Zinc (30 mg)	Nature Made USP (USA)
	D_3 (5000 IU)	Nature Made USP (USA)

B. Preparation of culture media.

Muller-Hinton agar, prepared according to FDA guidelines, is considered the optimal medium for cultivating a wide range of Pathogenic bacteria. Bacterial swabs were taken and cultured on culture media (Blood agar, Macconkey agar, Manitol salt agar) and cultured on Nutrient agar medium for purification. Then the bacterial diagnosis of those samples was carried out, which included microscopic diagnosis, observing the gram staining of those samples and distinguishing between gram-negative bacteria and gram-positive bacteria. Then the culture characteristics of the bacterial colonies such as their shape, size, color and texture were observed, and then biochemical tests were performed for the isolated samples such as mannitol fermentation test, catalase test, oxidase test, clotting test and IMVIC test . I used the API (Analytical Profile Index System) to diagnose the types of bacterial isolates after confirming them with the aforementioned preliminary biochemical tests. Preparation of bacterial inoculum, a. 3 to 5 of bacterial colonies were transfer to a tube of saline. b. The turbidity of tube was compared and adjusted to 0.5 McFarland turbidity standard using saline or broth. c. The plate of Mueller-Hinton agar was inoculated by dipping a sterile swab into the inoculum and the excess inoculum was removed. d. The plates were streaked by the swab all over the surface of the medium many times. Finally, allowed to dry.

C. Antibiotic susceptibility test

In vitro susceptibility tests were performed on Mueller-Hinton agar by disc diffusion method [22].

1) Streaking

Cotton swab (sterile) was dipped into the adjusted suspension and then rotated several times firmly on the inside wall of tube above the broth level to remove excess inoculum from the cotton swab.

All surfaces of a Mueller – Hinton agar plate was streaked with the dipping cotton swab. The striking surface of a ensure an even distribution of inoculum. As another step three rim of the agar was swabbed.

The petri dishes were Allowed to dry for time between 15-20 min at room temperature before the application of the antibiotic discs.

2) Application of Antibiotic Discs:

The antibiotic discs were dispensed onto the surface of the inoculated agar plate. Every disc pressed gently to ensure that will be complete contact with the agar surface via helping sterilized forceps.

The plates was inverted and placed in an incubator at 37˚C for 18-24 h. The diameter of growth inhibition zones around the discs on plates measured via using transparent ruler.

The results were compared with the tables of the CLSI [23].

Mueller– Hinton agar plate has been repeated two more times and the plate was rotated about 60˚ each time to

D. Statistical analysis

The data was analyzed using Excel software for Windows 2010. Descriptive statistics were utilized to analyze all data and calculated the Chi-square test to assess significant differences (p-value<0.0001) between the various parameters examined in this study.

III. Results and Discussion

To determine the efficacy of treatments, vitamin D3, and zinc on bacterial species, susceptibility testing was conducted. Results revealed that Azithromycin (21.5%) and Levofloxacin (17.7%, 15.9%) had lower sensitivity against Escherichia coli and *Staphylococcus aureus*, respectively. Meanwhile, *Streptococcus* showed high resistance to acyclovir and vitamin D3 (0.00%). Table (2) and Figure (1) indicated that Klebsiella was the most sensitive species among the others.

Table (2): Antibiotic susceptibility test against all types of bacteria

Names of treatments	*staphylococcus aureus (%)*	*Streptococcus (%)*	*Escherichia coli (%)*	*Klebsiella (%)*	2χ	df	Chi2 Pvalue
1-Azithromycin.	20 mm (21.05%)	30 mm 31.58%	20 mm 21.05%	20 mm)26.32%)	**55.37**	**12**	**<0.0001**
2-Acyclovir	10 mm (40.00%(	no effect)0.00%(	no effect)0.00%(	10 mm)60.00%)			
3-Levofloxacin	18 mm (15.93%(	40 mm 35.40%	20mm)17.70%)	18 mm) 30.97%(			
4-Zinc	20 mm)28.57%(	10 mm 14.29%	15 mm) 21.43%(	20 mm (35.71%(			
5-Vitamin	no effect) 0.00%(	no effect)0.00%(	5 mm 100.00%	no effect) 0.00%(			

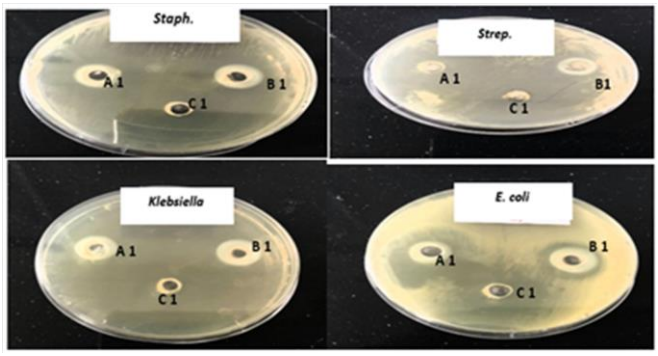

Figure (1): Antibiotic susceptibility test against all types of bacteria

The results in the second group which are shown in Table (3) reveals that Escherichia coli exhibited lower sensitivity to the zinc treatment solution compared to other species, particularly to the Azithromycin mixture with zinc (10.5%) and (15%) for the Acyclovir mixture with zinc, while Klebsiella demonstrated the highest sensitivity to the treatments. Figure (2) provides a visual representation of the results.

Table (3): Antibiotic susceptibility + zinc against all types of bacteria

Names of treatments	staphylococcus aureus (%)	Streptococcus (%)	Escherichia coli (%)	Klebsiella (%)	2χ	df	Chi2 Pvalue
1-Azithromycin+Zinc	20 mm)21.05%)	30 mm)31.58%)	10 mm (10.53%)	35 mm (36.84%)	**5.803**	**6**	**0.4456**
2-Acyclovir+Zinc	15 mm)28.30%)	10 mm)18.87%)	8 mm 15.09%	20 mm (37.74%(			
3-Levofloxacin+Zinc	20 mm (18.18%)	30 mm)27.27%)	20 mm 18.18%	40 mm (36.36%)			

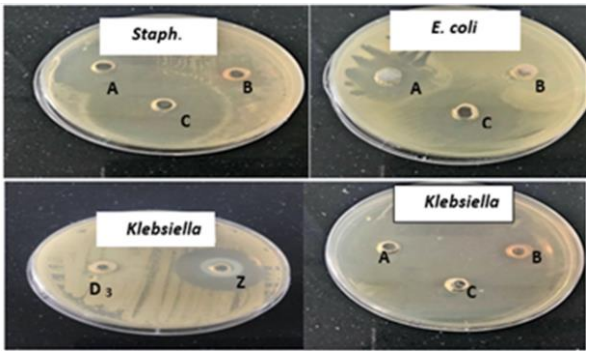

Figure (2): Anti biotic susceptibility + zinc against all types of

The results of the second groupdetailed in Table (4), examined the effects of vitamin D and treatments on bacterial species. Staphylococcus aureus displayed the highest resistance, as it was only affected by vitamin D and Levofloxacin (20%), while Streptococcus demonstrated the highest resistance to Acyclovir with D3 (0.00%). Escherichia coli displayed varying sensitivity levels to all treatment groups, whereas Klebsiella was the most sensitive to all treatments. Figure (3) illustrates the results.

Table (4): Antibiotic susceptibility test against all types of bacteria

Names of treatments	staphylococcus aureus (%)	Streptococcus (%)	Escherichia coli (%)	Klebsiella (%)	2χ	df	Chi2 Pvalue
1.Azithromycin+VitaminD3	no effect (0.00%)	10 mm (22.22%)	15 mm (33.33%)	20mm (44.44%)	**35.25**	**6**	**<0.0001**
2-Acyclovir+VitaminD3	no effect (0.00%)	no effect (0.00%)	10 mm (66.67%)	5 mm (33.33%)			
3-Levofloxacin+VitaminD3	15 mm (20.00%)	30 mm (40.00%)	15 mm (20.00%)	15 mm (20.00%)			

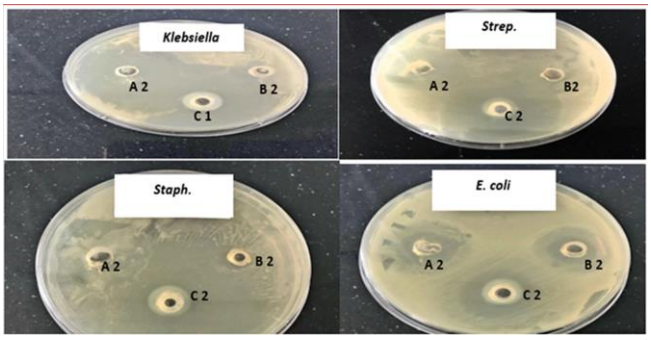

Figure (3): Antibiotic susceptibility + Vitamin D 3 against all types of bacteria

In general, it can be said that Azithromycin and Levofloxacin had lower susceptibility against *Escherichia coli and Staphylococcus aureus*, respectively. In contrast, *Streptococcus* showed high resistance to acyclovir and vitamin D3. From the tables and figure provided, it can be seen that *Klebsiella pneumonia* was the most sensitive species among other types of bacteria.

According to the results, Azithromycin demonstrated high efficiency against both types of bacteria, based on the findings from the previous study [24] with minimal impact when combined with zinc [25] The search results provide evidence to support this claim: A study on the early home administration of azithromycin with zinc supplementation found that zinc was efficient in preventing pneumonia and its therapeutic effects were consistent with evidence-based data [26] ,but reduced effectiveness when mixed with vitamin D [27]. In summary, the combination of azithromycin and vitamin D did not show a significant improvement in the treatment effect of azithromycin, leading to reduced effectiveness.

Different bacterial species exhibited varying levels of sensitivity to the antibiotics and supplements tested. Escherichia coli and Staphylococcus aureus showed less sensitivity to specific antibiotics. Streptococcus displayed high resistance to certain treatments, while Klebsiella was more sensitive among the species tested [28] [29] . Levofloxacin showed best results and remained unaffected by the addition of zinc or vitamin D. The previous search results provide evidence to support this claim: Levofloxacin, a fluoroquinolone antibiotic, can be affected by the presence of zinc and vitamin D. Zinc can cause a decrease in the absorption of Levofloxacin, resulting in a reduced serum concentration and potentially a decrease in efficacy [30]. Vitamin D has been shown to have a nephroprotective effect against Levofloxacin-induced renal damage. However, this does not directly affect the efficacy of Levofloxacin [31]. A study on the effect of acyclovir on microbial contamination found that Staphylococcus aureus proliferation was completely inhibited by acyclovir, indicating its impact on bacterial growth [32,33].

The previous studies have investigated the impact of vitamin D3 on bacteria. For example, a research article published in the journal "Infection and Immunity" in 2006 revealed that vitamin D3 can decrease the activity of tuberculosis bacteria in lung fluid cells. The study concluded that vitamin D3 can inhibit bacterial growth and enhance the creation of antimicrobial peptides in lung fluid cells [34], [14], [35]. A study published in [36] discovered that use of vitamin D3 alongside antibiotics can amplify their effectiveness against methicillin-resistant Staphylococcus aureus (MRSA) bacteria. The researchers noted adding vitamin D3 to standard antibiotics resulted in a greater ability

to eliminate MRSA bacteria in both cellular and laboratory animal studies. These findings align with previous research conducted by [37]. A study published in [38] concluded that vitamin D3 is a potent combatant against antibiotic-resistant *Escherichia coli* bacteria. The findings of the study revealed that addition of vitamin D3 to antibacterial treatments significantly enhanced their ability to eliminate resistant bacteria. Zinc supplementation has been shown to enhance the antimicrobial effects of certain antibiotics, particularly quinolone antibiotics like ciprofloxacin and azithromycin. This interaction can improve the effectiveness of these antibiotics against bacteria

However, taking zinc with tetracycline antibiotics can decrease the amount of tetracycline the body absorbs, potentially reducing their effectiveness [39,40]. To avoid this interaction, antibiotics should be taken at least 2 hours before or 4-6 hours after zinc supplements

IV. Conclusions

The study reveals that Azithromycin and Zinc significantly impact *Staphylococcus aureus*, while Azithromycin shows promising results against *Streptococcus* bacteria. Levofloxacin is particularly effective against *Streptococcus* bacteria compared to other antibiotics. Acyclovir's effect on bacteria can be enhanced with zinc. Vitamin D has limited efficacy, except against one strain of bacteria. Azithromycin and Zinc have strong effects on *Staphylococcus aureus*, while Levofloxacin has more effect on *Streptococcus* bacteria. Acyclovir's effect is enhanced with zinc.

Conflict of interest

Authors declare that they have no conflict of interest.

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