Antibacterial Activity of Water Soluble and Fat-Soluble Vitamins against Drug Resistance Clinical Bacteria *Klebsiella Pneumonia*  

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**ABSTRACT**  
**Background:** Vitamins play an important role in enhancing immunity, which helps the body fight various infections. **Aim:** This study investigates the antimicrobial activity of Biotin (Vitamin B7) and Ascorbic Acid (Vitamin C) against clinically significant microorganisms, specifically *Klebsiella pneumoniae*. We explored their potential as agents for disrupting protein biotinylation and the mechanisms underlying their antimicrobial effects. **Materials and Methods:** Clinical microorganisms were obtained from the Clinical Microbiology Lab in Coimbatore, India and cultured to match the MacFarland standard turbidity. The Kirby-Bauer Method was used to assess the antimicrobial activities of Biotin, Ascorbic Acid, and other vitamins against *K. pneumoniae*. The Minimum Inhibitory Concentrations (MIC) of Biotin and Ascorbic Acid were determined using a serial dilution method. Transmission Electronic Microscopy (HR-TEM) was used to visualise the antimicrobial effects of Biotin and Ascorbic Acid on *K. pneumoniae*. **Results:** The results showed antimicrobial activity of Biotin and Ascorbic Acid against *K. pneumoniae*, with zones of inhibition of 14 and 12 mm, respectively. The MIC values for Biotin and Ascorbic Acid were 62.5 and 125 µg/mL, respectively. HR-TEM analysis revealed significant morphological alterations in the treated bacterial cells compared with those in untreated cells. **Conclusion:** This study provides insights into the antimicrobial potential of Biotin and Ascorbic Acid, and highlights their mechanisms of action. Unlike traditional antibiotics, these vitamins demonstrate promising antimicrobial effects and have potential applications as combination therapies or adjuncts to conventional treatments. **Keywords:** Anti-microbial resistance, Anti-microbial activity, Biotin (B7), Ascorbic acid (C), MIC.  

**INTRODUCTION**  
AMR (antimicrobial resistance) arises in microorganisms making them less susceptible to treatments.[1] This phenomenon renders infections more challenging to manage, elevates the potential for disease transmission, and increases the likelihood of severe health complications and mortality. As a result of the emergence of resistance, the efficacy of antibiotics has diminished and other antimicrobial treatments diminishes, leading to progressively more intricate or even insurmountable infections.[2] The rise and propagation of drug-resistant microorganisms, which have developed novel resistance mechanisms, poses an ongoing threat to their ability to effectively manage common infections. A particularly
concerning issue is the quick international expansion of multi resistant bacteria, often mentioned to as “superbugs”, capable of infection causes that difficult to treat with exiting antimicrobial medications, including antibiotics. However, pipelines for new antimicrobial drugs are currently insufficient. WHO In 2019 reported 32 antibiotics at various stages of clinical development to combat priority pathogens; however, only six of these were deemed innovative.[9] Access to high-quality antimicrobial drugs is a critical concern. A shortage of antibiotics affects countries across all developmental stages, significantly affecting their healthcare systems. As the global spread of drug resistance continues, antibiotics are progressively losing their effectiveness, leading to formidable infections and loss of life.[6] The urgent development of new antibiotic treatments is necessary, particularly for addressing gram-negative bacteria's existing resistance to carbapenem antibiotics, as highlighted in the WHO priority pathogen list. However, it is important to note that, unless there is a shift in the way antibiotics are currently used; new antibiotics will encounter the same fate as their predecessors and eventually become ineffective. The economic and healthcare-related costs of AMR are substantial, affecting national economies and healthcare systems by reducing productivity owing to extended treatment and the need for more intricate and costly care.

Without efficacious strategies to stop and sufficiently treat infections and improve access to both already present and novel anti-microbial medications, there will be a surge in the number of individuals experiencing treatment failure or succumbing to infections. Essential medical procedures such as surgery, cancer treatments such as chemotherapy, and organ transplants will become more precarious.

Aqueous soluble vitamins are quickly dissolved and fatly observed in the body. They are necessary for sustaining good health because they are vital for many physiological functions. Aqueous soluble vitamins include Vitamin C and B. Although aqueous-soluble vitamins are typically not known for their direct antibacterial activity, some play important roles in supporting the immune system, which indirectly contributes to the body’s defence against bacterial infections.[5,6]

Vitamins A, D, E, and K are fat-soluble vitamins that show energetic functions in various physiological processes. Although their potential antibacterial activities have not been extensively studied, they can contribute to defence against bacterial infections through various mechanisms. Vitamin A maintains healthy mucosal surfaces, bolsters immune responses, and promotes the production of antimicrobial peptides. Vitamin D modulates the immune system, enhancing defence mechanisms against infections and regulating inflammation.[7] Vitamin E antioxidant properties protect cell membranes from oxidative damage, indirectly supporting immune function, and countering bacterial infections. Vitamin K antibacterial role is less established, but it may impact immune-related protein expression and promote a healthy balance of gut bacteria. [8] It is important to consult a healthcare professional before using fat-soluble vitamins or supplements owing to their potential antibacterial properties, as higher dose can lead to toxicity. Evaluation of antibacterial activity vitamins against clinically resistant microorganisms.

MATERIALS AND METHODS

Materials

Muller Hinton Agar (MHA), Nutrient Broth (NB), Clinical Bacteria (Klebsiella Pneumonia), riboflavin (B2), biotin (B7), Ascorbic acid (C), and Cobalamin (B12), A, D, and E vitamins.

Culture Media and Clinical Micro-organisms

The antibacterial effects of the vitamins were tested against the clinically relevant pathogen K. pneumoniae, which was obtained from the Clinical Microbiology Laboratory in Coimbatore, India. They were sub-cultured individually for 24 hr at 37°C in 5 mL sterile nutritional broth and adjusted to meet the turbidity of the MacFarland standard.

Agar-Well plate method

Riboflavin (B2), biotin (B7), ascorbic acid (C), and Vitamins A, D, and E were evaluated for anti-bacterial activity by agar well plate method. Sterile double-strength MHA medium (7.6 g in 100 mL) (Himedia) was prepared and clinical microbial cultures were coated MHA plate surface using sterile cotton swabs. 100 µL each of riboflavin (B2), biotin (B7), ascorbic acid (C), and Vitamins A, D, and E were aseptically applied in duplicate to wells created on agar using a sterile borer. Ciprofloxacin (10 g/mL) was added to the agar wells as a positive control. After placing the culture plates in a refrigerator for 30 min to facilitate vitamin permeation into the agar, the plates underwent a 24 hr incubation period at 37°C. The evaluation of antibacterial activity was conducted using a zone reader from HiMedia.

MIC of biotin (B7) and ascorbic acid (C)

The antibacterial activity was conducted followed by Moussa et al.[9] with some modifications. Different concentrations of Control and biotin (B7), ascorbic
acid (C) different concentration from 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/mL was added 96 well cell culture plates along with 100 µL of the test microorganism. 96 well plates incubated for 24 hr and observed MIC.

**HR-TEM to examine antimicrobial effects of biotin (B7), Ascorbic acid (C)**

Bacteria treated with biotin (B7) and ascorbic acid (C) were centrifuged, cleaned twice with sterile water, and resuspended in PBS. After being fixed for 2 hr at room temperature in 2.5 percent glutaraldehyde, the cells were dehydrated with alcohol, dried with hexamethyldisilazane (HMDS), and coated with gold. The effects of biotin (B7) and ascorbic acid (C) on bacterial cells were examined using HR-TEM (TESCAN). The samples were prepared for HR-TEM analysis from harvest to centrifugation. A drop of the suspension was placed on a copper grid and dried. Using a JEM-2100PLUS TEM, the grids were operated at 200 kV software (JEOL Ltd., Tokyo, Japan).

**RESULTS**

**Antimicrobial activity**

The anti-microbial activities of biotin (B7) and ascorbic acid (C) against selected clinical *K. pneumoniae* were evaluated using the agar-well diffusion method on plates bearing a lawn of the appropriate clinical microorganism. Zone diameters were noted and Table 1 and Figure 1 one reported the antibacterial activity results.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Vitamins</th>
<th>Zone of Inhibition (mm) (n=4)</th>
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<tbody>
<tr>
<td>1</td>
<td>Riboflavin (B2)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Biotin (B7)</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>Ascorbic acid (C)</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin A</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Vitamin E</td>
<td>-</td>
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<tr>
<td>6</td>
<td>Vitamin D</td>
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**Figure 1: Antimicrobial activity of Riboflavin (B2), Biotin (B7), Ascorbic acid (C), Vitamin A, Vitamin E and Vitamin D and standard drugs against *K. pneumoniae*.**
Determination of MIC for Biotin (B7) and Ascorbic acid (C) against clinical *K. pneumonia* using serial dilution method

The MIC of biotin (B7) and ascorbic acid (C) for *K. pneumonia* are listed in Table 2. Biotin (B7) and ascorbic acid (C) showed antibacterial activity against clinical *K. pneumonia* at 62.5 µg/mL and 125 µg/mL. Biotin is a water-soluble B vitamin that showed an important function in different body metabolic like carbohydrate, fat, and protein metabolism. It is an essential nutrient for humans and is often found in foods, such as eggs, nuts, and certain vegetables. In your statement, biotin has exhibited antibacterial activity against clinical *K. pneumonia* strains at a concentration of 62.5 µg/mL. This suggests that at 62.5 µg/mL concentration, biotin was effective in inhibiting the growth or viability of *K. pneumoniae*. Ascorbic acid (Vitamin C) is a well-known antioxidant that acts as a crucial important function in the immune system and in overall health. Vitamin C presents various vegetables and fruits such as citrus fruits, strawberries, and bell peppers. Ascorbic acid showed antibacterial activity against clinical *K. pneumonia* strains at a concentration of 125 µg/mL. This means that, at this concentration, vitamin C was effective in inhibiting the growth of *K. pneumoniae*. It is important to note that the antibacterial activities of biotin and Vitamin C may have implications in the fields of microbiology and healthcare. Researchers often investigate the potential antibacterial properties of various compounds, including vitamins and other natural substances. These findings may contribute to the development of novel treatments and therapies for bacterial infections.

**High-resolution transmission electron microscopy of biotin (B7)- and ascorbic acid©-treated *K. pneumoniae***

Morphological alterations, including cellular contraction and cell wall disintegration, were noted in bacterial cells subjected to biotin (B7) and ascorbic ac©(C) treatment during HR-TEM analysis. In contrast, untreated cells exhibited a typical morphology (Figure 2 for visual representation).

**DISCUSSION**

The study presents interesting findings on the antimicrobial activities of biotin (B7) and ascorbic acid (C) against clinical *K. pneumoniae*. The agar-well diffusion method revealed significant zones of inhibition for both biotin (14 mm) and ascorbic acid (12 mm), indicating their antibacterial effects. The MIC further emphasized the potency of biotin at 62.5 µg/mL and ascorbic acid at 125 µg/mL against *K. pneumoniae*. Biotin, a water-soluble B Vitamin with essential roles in metabolic processes, demonstrated antibacterial efficacy at a relatively low concentration. Soares da Costa TP and his colleagues studied the anti-bacterial activity of the biotin-based analogues.[10] The biotin-based chemical analogues are showing good antibacterial activity against MRSA with 8 µg/mL MIC concentration.[11] Our study found that biotin itself showed anti-bacterial activity against *K. pneumoniae*. The study emphasizes the importance of biotin in human nutrition and highlights its additional role in inhibiting bacterial growth. Ascorbic acid, known for its antioxidant properties and vital functions in the immune system, exhibited antibacterial activity at 125 µg/mL. The study underscores the potential of Vitamin C as a therapeutic agent against *K. pneumoniae*. Given its widespread availability in fruits and vegetables,

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Microorganism</th>
<th>Minimum inhibitory concentration (n = 4)</th>
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<tr>
<td></td>
<td>Biotin (B7)</td>
<td>Ascorbic acid (C)</td>
</tr>
<tr>
<td>1</td>
<td><em>K. pneumonia</em></td>
<td>62.5 µg/mL 125 µg/mL</td>
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the findings open avenues for exploring natural sources in the development of anti-bacterial treatments.

The High-Resolution Transmission Electron Microscopy (HR-TEM) analysis provided visual insights into the morphological alterations induced by biotin and ascorbic acid in K. pneumoniae cells. The observed cellular contraction and cell wall disintegration suggest a direct impact on bacterial structure. The K. pneumoniae cells were lysis and cells were broken. Untreated bacterial cells appear smooth and round. This information is vital in understanding the mechanisms of antibacterial action, laying the groundwork for further research and development.

The antimicrobial activity and its mechanisms of Biotin (Vitamin B7) and Ascorbic Acid (Vitamin C) involve distinct biochemical pathways that contribute to their effects against microorganisms. Biotin (Vitamin B7) is a water-soluble B-Vitamin that is essential for several metabolic functions. B7 Vitamin acts as essential enzyme co-factor of various enzymes involved in energy metabolism, including carbohydrate, fat, and protein metabolism. Biotins can indirectly affect microbial growth and viability by affecting these pathways. Biotin-dependent enzymes contribute to cell wall synthesis in certain microorganisms. The inhibition of these enzymes may disrupt the integrity of the cell wall, leading to cell lysis and reduced bacterial viability. Biotin-producing bacteria may inhibit the growth of biotin-dependent pathogens by competing for available biotin resources. This mechanism could limit the growth of pathogenic microorganisms in environments where biotin availability is restricted.[12]

Ascorbic Acid (AA) is a well-known antioxidant that has a range of biological functions. Its antimicrobial activity mechanisms stem from its ability to modulate the immune response and influence bacterial growth. Ascorbic acid enhances immune system function by promoting the production and activity of immune cells, such as phagocytes and lymphocytes. A strong immune response can aid the clearance of bacterial infections. Ascorbic acid can generate large amounts of reactive oxygen species. Reactive oxygen species can cause oxidative stress in bacteria, injure cellular components, and inhibit bacterial growth.

Ascorbic acid can chelate iron, a crucial nutrient for bacterial growth, and limits its availability to bacteria by sequestering iron, potentially restricting its ability to proliferate. The effect of ascorbic acid on pH can influence bacterial growth. The lowered pH due to ascorbic acid may create an environment unsuitable for bacterial survival and reproduction. The ability of ascorbic acid to disrupt biofilms, which are protective matrices formed by bacteria, can enhance the susceptibility to immune responses and antibiotics. [13]

Very important to understand the antimicrobial mechanism of action of Biotin and Ascorbic Acid have not been studied as extensively as those of traditional antibiotics. Their effects can vary depending on factors, such as concentration, micro-organism type, and the overall environment. As such, these vitamins are not typically used as primary antimicrobial agents but might have potential applications in combination therapies or as adjuncts to conventional treatments.

Mumtaz and his research team conducted a study to investigate the antibacterial properties of Vitamin C under diverse environmental conditions. They explored the effects of vitamin C at different temperatures (4, 37, and 50°C) and pH levels (3, 8, and 11). This investigation involved testing different concentrations of Vitamin C (5-20µg/mL) and utilising the agar well diffusion method. This study revealed that Vitamin C effectively inhibits Gram-positive and Gram-negative bacteria, resulting in inhibition zones with precise measurements. 25.3±0.9 mm (B. lichenformis), 22.0±0.6 mm (S. aureus) 19.3±0.3 mm (B. subtilis), 27.67±0.882 mm (P. mirabilis), 21.33±0.9 mm (K. pneumoniae), 18.0±1.5 mm (P. aeruginosa) and 18.3±0.3 mm (E. coli).[8] We observed that biotin and Vitamin C showed antibacterial activities of 14 and 12 mm, respectively, against K. pneumoniae. When compared to the Mumtaz research results, the observed decrease in the zone of inhibition can be related to the prevalence of drug-resistant bacterial strains. Furthermore, it is worth mentioning that our study is the first to report the antibacterial activity of biotin, which provides novel insights into the field of microbiology and underscores the need for continued research into alternative treatments for drug-resistant K. pneumoniae infections.

**CONCLUSION**

Antimicrobial activity of biotin (Vitamin B7) and ascorbic acid (Vitamin C) against clinical strains of K. pneumoniae. The well plate method was employed to study their inhibitory effects, and the results revealed that biotin showed a 14 mm zone of inhibition, whereas ascorbic acid showed a 12 mm zone of inhibition against K. pneumoniae. These findings suggested that both Biotin and Ascorbic acid have potential antimicrobial properties against this clinical microorganism. The MIC of biotin and ascorbic acid were measured using the serial dilution method. The broth dilution method was used to estimate of MIC of vitamins and the MIC values were found to be 62.5 µg/mL for biotin and 125 µg/mL for ascorbic acid respectively. These MIC values provide insight into the concentration at which these vitamins effectively inhibit bacterial growth. The study also utilised the morphological changes generated by Biotin and Ascorbic acid treatment in K. pneumoniae cells using HR-TEM. The
experimental changes, such as cellular contraction and cell wall disintegration, corroborate the antibacterial actions of these vitamins against this bacterium. Compared to untreated cells, which displayed a typical morphology, the treated cells exhibited noticeable structural changes, indicating a potential mechanism of action by which Biotin and Ascorbic acid exert their antimicrobial effects. Overall, this study contributes to our understanding of the antimicrobial properties of Biotin and Ascorbic acid against the clinical strains of *K. pneumoniae*. Further research should explore the specific mechanisms through which these vitamins exert their antimicrobial effects, potentially paving the way for novel therapeutic strategies to combat bacterial infections.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**ABBREVIATIONS**


**SUMMARY**

This study investigated the antimicrobial activity of biotin (Vitamin B7) and ascorbic acid (Vitamin C) against clinical strains of *K. pneumoniae*. Using the agar-well diffusion method, both vitamins exhibited zones of inhibition of 14 mm for biotin and 12 mm for ascorbic acid, indicating their potential antimicrobial properties. The MIC values were determined 62.5 µg/mL for biotin and 125 µg/mL for ascorbic acid, highlighting their effective concentrations. High-Resolution Transmission Electron Microscopy (HR-TEM) revealed morphological changes in treated *K. pneumoniae* cells, including cellular contraction and cell wall disintegration, supporting the antibacterial actions of biotin and ascorbic acid. These findings contribute to the understanding of the potential mechanisms by which these vitamins inhibit bacterial growth and offer insights into their role in combating microbial infections.

**REFERENCES**