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Original article

Ascorbic acid-mediated control of microbial infections in blood

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Abstract

Background: Ascorbic acid (AA) is a naturally occurring organic compound found in animals and plants with antimicrobial and antioxidant potential; it functions as a redox buffer that can reduce and neutralize reactive oxygen species.

Objective: This study aimed to investigate AA-mediated control of microbial infection in blood through oxidation-reduction reactions, with and without AA.

Methods: Different genera of bacteria (*Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae*, *Acinetobacter baumannii,* and *Pseudomonas aeruginosa*) and a fungus *(Candida albicans*) were cultured in plasma from individuals with different ABO groups, followed by measurement of oxidation-reduction reactions using cyclic voltammetry in the presence and absence of AA.

Results: An AA concentration of 100 g substantially affected microorganisms, *K. pneumoniae* (25 mm), *S. aureus*, and *P. mirabilis* (24 mm), followed by *A. baumannii*, *P. aeruginosa*, *E. coli* , and *C. albicans* (19, 18, 17, and 15 mm, respectively). High antioxidative results were recorded in the presence of AA in plasma compared with its absence in group A plasma infected with *A. baumannii*, with the difference between the oxidation current peaks of infected blood with other microorganisms treated with and without AA (ΔIpa). The results showed that ΔIpa=10 for *P. mirabilis* was less affected, with ΔIpa=2 in plasma from blood group B. *A. baumannii* was highly affected with ΔIpa=13; *P. aeruginosa* was less affected with ΔIpa=2 in plasma from blood group AB. *A. baumannii* was highly affected with ΔIpa=15; *K. pneumoniae* was less affected with ΔIpa=1 in plasma from blood group O. *C. albicans* was highly affected with ΔIpa=31, and *K. pneumoniae* was less affected with ΔIpa=2.

Conclusion: The study found that AA inhibits the growth and activity of various pathogenic microorganisms when added to infected blood samples. This effect was observed in blood samples infected with bacteria such as *A. baumannii* and *P. mirabilis*, as well as the fungus *C. albicans.*

Keywords: Ascorbic acid, blood groups, cyclic voltammetry, microorganisms, redox reaction.

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Bacteremia results from viable bacteria in the bloodstream and may affect healthy individuals and those with underlying comorbidities. Bacteremia is considered an urgent health burden.[1] Asymptomatic bacteremia can occur during normal daily activities, such as during oral hygiene, and after minor medical procedures. In a healthy individual, this clinically benign infection is transient and causes no additional consequences.[2] However, bacteremia can progress to a bloodstream infection that may present with a spectrum of clinical syndromes, such as septicemia, when the immune response malfunctions or becomes exhausted.[3] Furthermore, septic shock, systemic inflammatory response syndrome, sepsis, and multiple organ dysfunction syndrome may result from untreated bacteremia.[4] Microbes, including bacteria and fungi, can cause bloodstream infections. Bacteria are responsible for 90% of bloodstream infections, including gram-positive and gram-negative species.[5] Top bacterial species that cause bacteremia include *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter* species, *Enterobacter* species, *Enterococcus faecalis*, *Proteus* species, *Streptococcus pneumoniae*, and *Salmonella* species.[6-8] Respiratory tract and indwelling catheters, especially central venous catheters, are the most frequent sources of infections in hospitalized patients. Moreover, untreated urinary tract infections are mostly caused by community-acquired bacteremia. Soft tissue and intra-abdominal infections are uncommon but are more likely to occur in postoperative surgical settings.

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S. aureus is the most common gram-positive associated cause of bacteremia, whereas the most frequent gram-negative cause *is E. coli*.[9]

The alarming increase in antibiotic resistance has created a need for non-conventional approaches to combat this problem. Therefore, identifying effective antimicrobial agents to combat these infections is an important avenue of research. One compound that has shown promising results is ascorbic acid (AA), or vitamin C. Previous research has shown AA's antimicrobial potential against a range of pathogens, including bacteria, viruses, and fungi. Proposed mechanisms include disruption of microbial cell membranes, generation of reactive oxygen species (ROS), and interference with nutrient absorption and metabolic processes.[10, 11] While researchers have studied the antimicrobial effects of AA in cell culture and animal models, research on its effects in infected human blood samples remains limited. Electrochemical techniques, such as cyclic voltammetry (CV), provide a powerful tool for studying the interactions between AA and microorganisms in a complex biological matrix such as blood. When different concentrations of AA are added to mercury, oxidation peaks increase due to the formation of the hemolytic complex in blood. This process occurs when consuming seafood with lemon juice (a source of AA), which can be fatal when seafood has high mercury ion content.[12] Although oxidative stress plays an important role in the pathophysiology of many diseases, neither oxidative stress nor antioxidants can be accurately measured for use in clinical settings. CV is an electrochemical method that is widely used in industrial and research settings to assess redox status. Recently, it has been used to assess the antioxidant state of live biological samples. This work aims to provide basic information for those who intend to use CV to measure antioxidants in biological samples in a clinical setting. Voltammetric techniques can identify numerous biological compounds, including AA, and measure their trace concentrations. Although AA is electrochemically active, electrode-surface issues make it challenging to measure directly with most carbon and transition-metal electrodes. Moreover, investigation of potential interference from multivitamin tablet components indicated specific selectivity toward AA and no electrochemical response to the other components. Treatment and management of bacteremia primarily depend on identifying the source of infection. A recent study suggested that voltammetric techniques could identify and quantify small concentrations of many biological molecules, such as L-ascorbic acid. L-ascorbic acid is electrochemically active, but most transition-metal and carbon electrodes struggle to quantify it directly because of electrode-surface challenges. AA may be used as a natural preservative in cheese because it inhibits artificially *Salmonella*-contaminated soft cheese.[13] Furthermore, the lack of undesirable side effects has made the use of AA a promising antibiotic-independent treatment strategy to combat many microbes.[14] Hence, this study aimed to investigate the AA-mediated control of blood-borne microbial infections by measuring oxidation-reduction reactions using CV.

Materials and methods

Materials

Seven microorganisms, including six bacterial species: *S. aureus, Proteus mirabilis, E. coli, K. pneumoniae, A. baumannii*, and *P. aeruginosa*, plus the fungus *Candida albicans*, were activated in brain heart infusion broth, blood agar, MacConkey agar, and Sabouraud dextrose (for the fungus). We previously identified all bacterial isolates using the VITEK® 2 GN gram-negative and gram-positive bacteria diagnostic kit (BioMérieux, France). We obtained these isolates from the Research Unit in the College of Health and Medical Techniques. AA was obtained from Technicon Chemical Company (Tournay, Belgium). Blood (from the four ABO groups) was obtained from healthy volunteers and used in the experimental tests. Plasma was obtained by centrifugation of the blood samples at 3000 rpm for 5 min. Bacterial suspension was prepared from the above organisms by mixing with 1 mL of distilled water using a vortex and adjusted to 1×10^8 CFU/mL using 0.5 McFarland standards. Then, 10 µL of bacterial suspension was mixed with 1 mL of plasma (1:100 dilution), followed by incubation at 37°C for 24 h. Next, 1 mL of cultured plasma was diluted with 9 mL of distilled water and measured using a CV (first reading). Then, AA was added, and the sample was measured using a CV (second reading). A plasma control without cultured bacteria was also included as a blank control.[15]

AA inhibition concentration determination

Agar diffusion was performed by making holes in Mueller-Hinton agar using a sterile Pasteur pipette. The cultured organisms were adjusted to 0.5 McFarland standards, the hole was filled with 0.1 mL of AA, and the plates were left to diffuse at room temperature for 2 h. The plates were then incubated at 37ºC for 18 h, followed by measurement of the inhibition zones.

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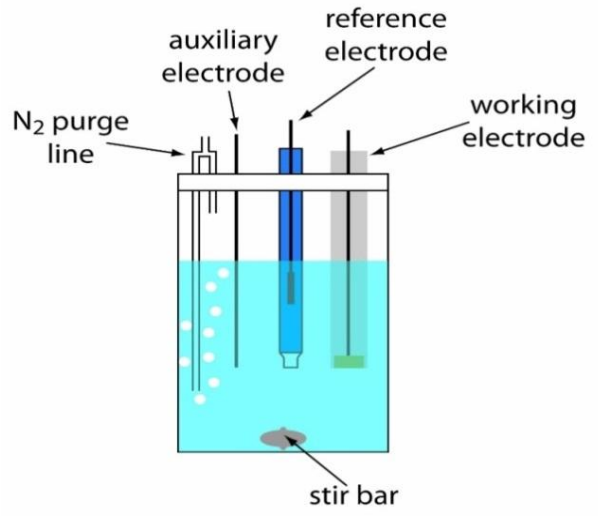


Figure 1. Cyclic voltammetric cell.

Ethical approval

The Medical Ethics Committee of Middle Technical University reviewed and approved this study IRB reference no:MCE 265.

CV study

An EZstat (potentiostat/galvanostat, NuVant Systems Inc., United States) was used in all experiments. All CV experiments used a glassy carbon electrode (GCE) as the working electrode, Ag/AgCl (3 M KCl) as the reference electrode, and a Pt wire (1 mm diameter) as the counter electrode **(Figure 1)**. All electrodes were connected, and we applied three electrical potentials using computer-based electroanalytical measurement software. We cleaned the GCE surface by polishing with alumina solution, followed by aqueous sonication for approximately 10 min to remove impurities before using it in the CV cell.[15]

Three electrodes (modified CNT/GCE as the working electrode, Ag/AgCl as the reference electrode, and Pt wire as the counter electrode) were placed in a 10-mL black tea solution in a voltammetric quartz cell (volume 15 mL). The three electrodes were connected to potentiostats, and results were obtained via periodic voltammetry using a computer.[16]

Results

Isolated microorganisms

Pure isolated colonies of *K. pneumoniae*, *E. coli, P. mirabilis,* and *A. baumannii* were inoculated onto MacConkey agar; *S. aureus* and *P. aeruginosa* onto blood agar; and *C. albicans* onto Sabouraud dextrose agar, then incubated at 37°C for 24 h **(Figure 2)**.

Biological effects observed with AA

The results of using different AA concentrations (25, 50, and 100 g) affected the various microorganisms, as demonstrated by high inhibition zones (IZ) with an AA concentration of 100 g in all microorganisms used in the study. The IZ of *K. pneumoniae* was 25 mm, followed by *S. aureus* and *P. mirabilis* (24 mm), and then by *A. baumannii, P. aeruginosa, E. coli,* and *C. albicans* (19, 18, 17, and 15 mm, respectively) **(Figure 3).**

Culturing of microorganisms in different ABO blood groups

Several bacterial species (*S. aureus, P. mirabilis, E. coli, K. pneumoniae, A. baumannii,* and *P. aeruginosa*) and a fungus (*C. albicans*) were cultured in different ABO blood group samples. Then, CV was used to measure oxidative stress (Ipa) in the infected blood before and after treatment with AA as an antioxidant.

Effect of AA on the oxidation of ABO blood group A inoculated with various microorganisms

In this *in vitro* study, different bacterial species (*S. aureus, P. mirabilis, E. coli, K. pneumoniae, A. baumannii, P. aeruginosa*) and a fungus (*C. albicans*) were cultured in blood group A plasma, and the oxidation-reduction current peaks were analyzed by CV. The results showed a strong antioxidative effect in the presence of AA compared with its absence (without AA). The following equation was used to calculate the ΔIpa:

ΔIpa = Ipa without AA – Ipa with AA

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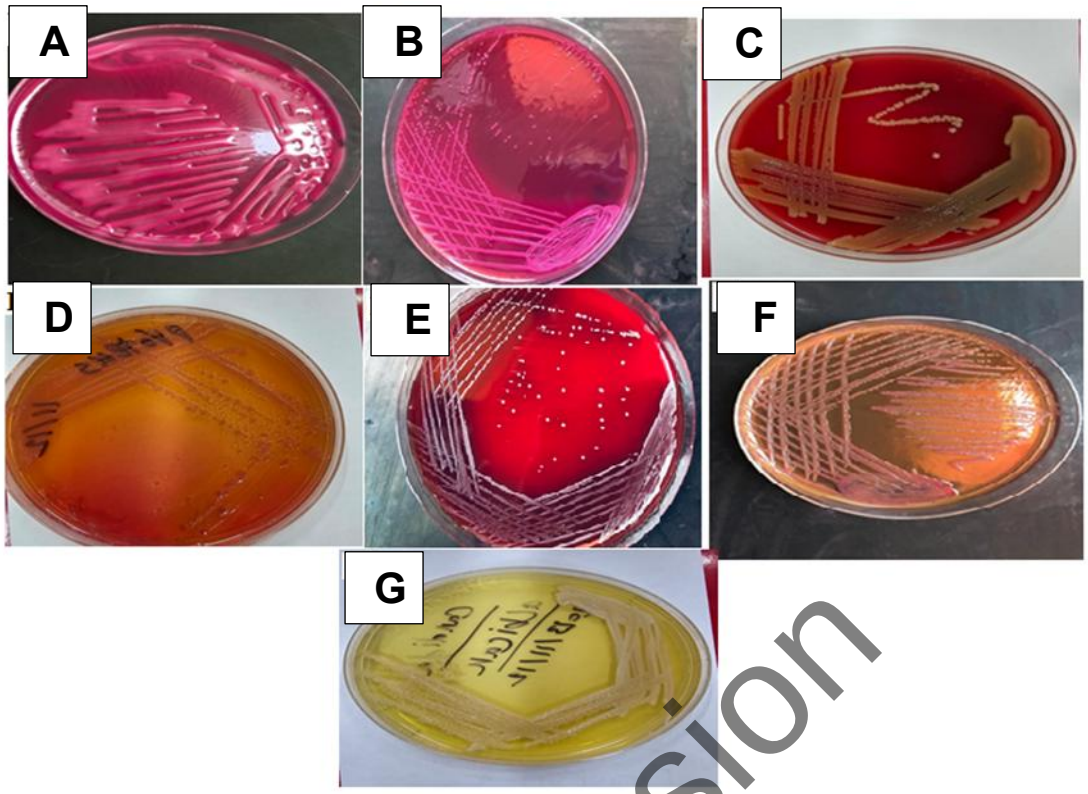
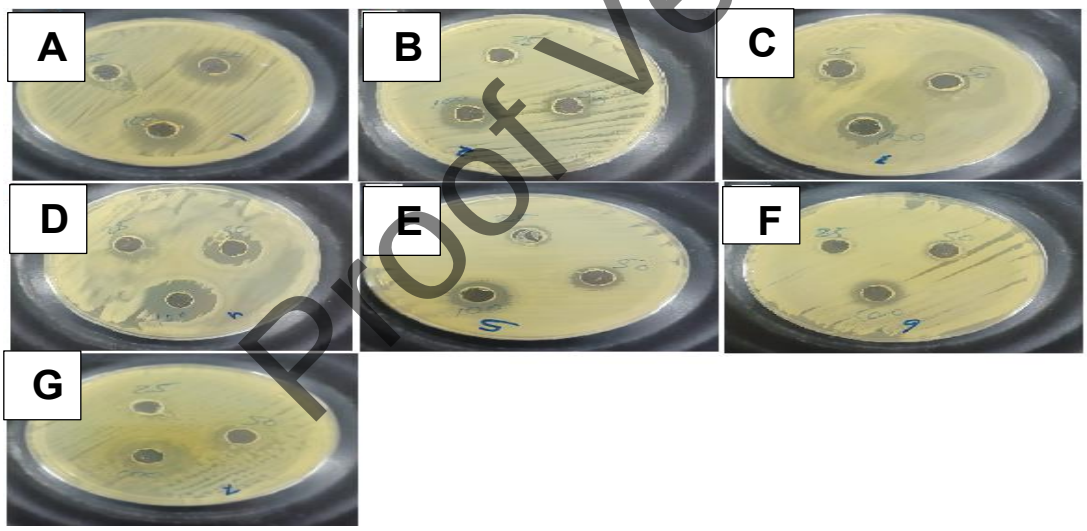


Figure 2. Different cultures of microorganism **(A)** *K. ppneumoniae* on MacConkey, **(B)** *E. coli* on MacConkey agar, **(C)** *S. aureus* on blood agar, **(D)** *P. mirabilis* on MacConkey agar, **(E)** *P. aeruginosa* on blood agar, **(F)** *A. baumannii* on MacConkey, **(G)** *C. albicans* on Sabouraud dextrose agar, all incubated at 37°C for 24 h.

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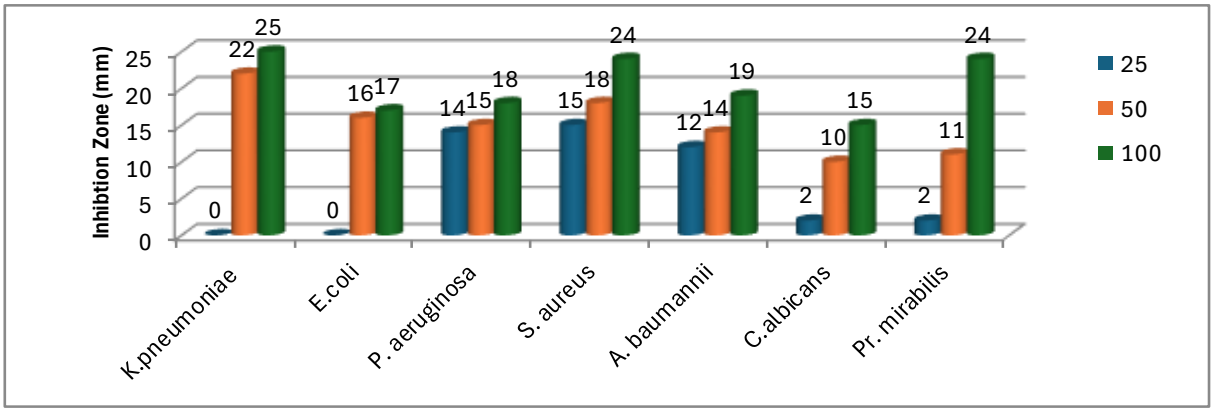


Figure 3. Inhibition Zone of different microorganisms **(A)** *K. pneumoniae*, **(B)** *E. coli,* **(C)** *P. aeruginosa*, **(D)** *S. aureus,* **(E)** *A. baumannii,* **(F)** *C. albicans,* **(G)** *P. mirabilis* affected by ascorbic acid concentration (25, 50, 100 mg), all incubated at 37°C for 24 h.

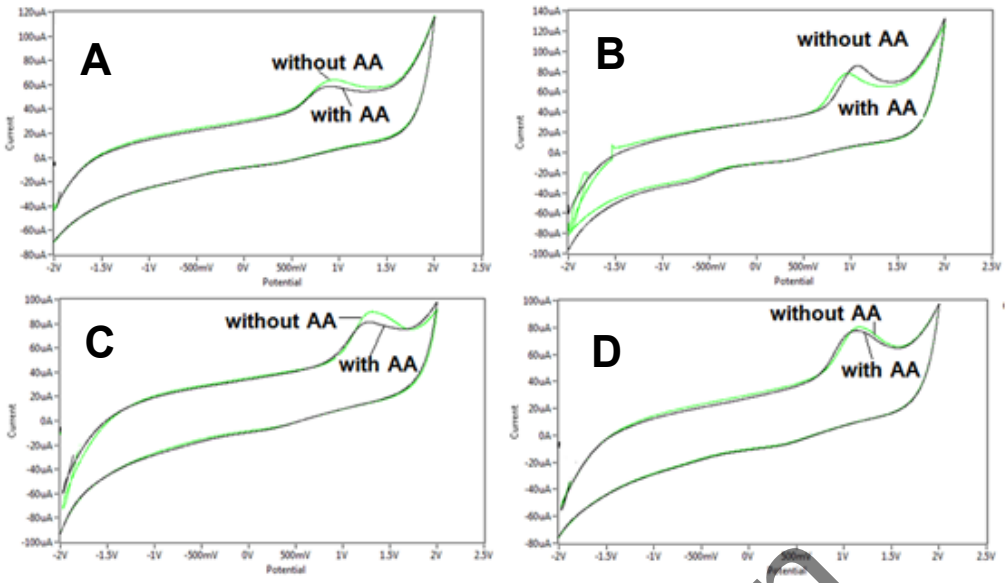


Figure 4. Cyclic voltammogram of different bacteria in ABO group A with and without AA. **(A)** *C. albicans*, **(B)** *E. coli*, **(C)** *A. baumannii*, and **(D)** *P. mirabilis*.

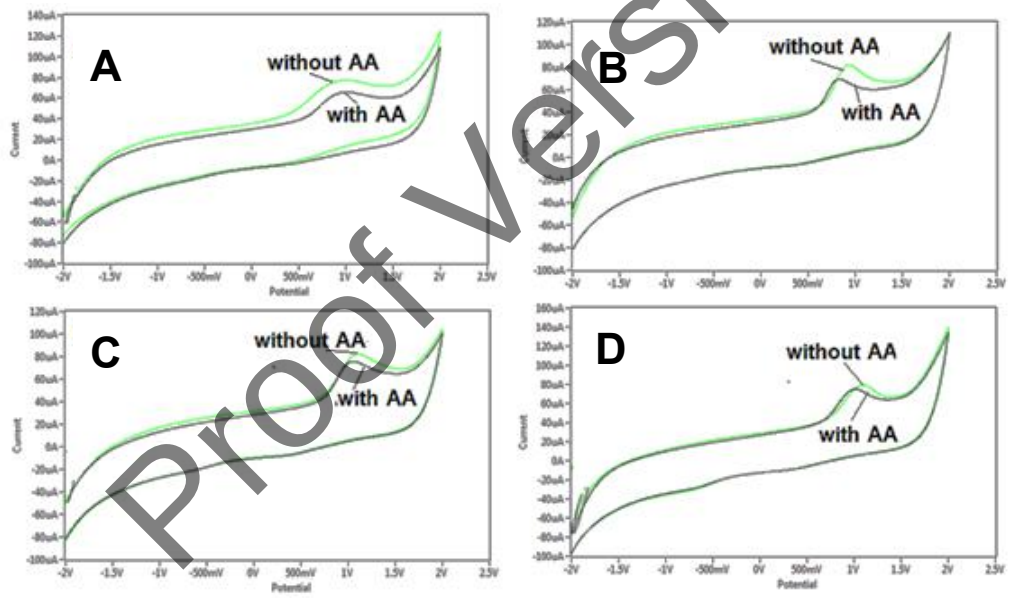


Figure 5. Cyclic voltammogram of different bacteria in blood group B with and without AA. **(A)** *C. albicans*, **(B)** *A. baumannii*, **(C)** *P. mirabilis*, and **(D)** *E. coli*.

A. baumannii was the most affected bacterium (ΔIpa = 10), followed by *E. coli* (ΔIpa = 9), *S. aureus* (ΔIpa = 8), *C. albicans* (ΔIpa = 6), *P. aeruginosa* (ΔIpa = *5), K. pneumoniae* (ΔIpa = *4),* and *P. mirabilis* (ΔIpa=2) **(Figure 4).**

Effect of AA on the oxidation of ABO Group B inoculated with various microorganisms

When the microorganisms under study were cultured in group B plasma, the findings revealed strong antioxidative results in the presence of AA compared with the test conditions without AA. *A. baumannii* was most affected (ΔIpa = 13), followed by *C. albicans* (ΔIpa = 11), *K. pneumoniae* (ΔIpa = 6), *E. coli* (ΔIpa = 4)*, S. aureus* (ΔIpa = 4), and *P. mirabilis* (ΔIpa = 3), and lastly *P. aeruginosa* (ΔIpa = 2) **(Figure 5)**.

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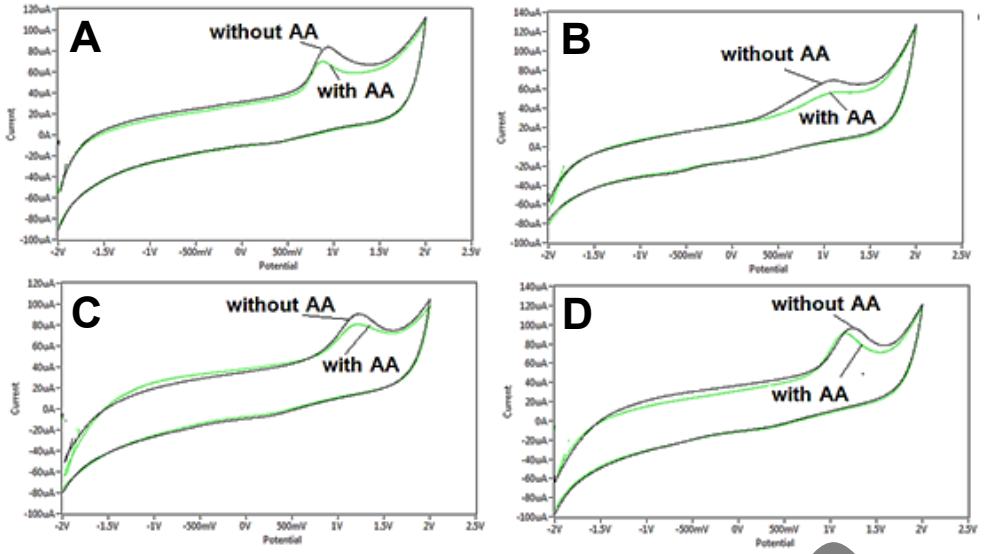


Figure 6. Cyclic voltammogram of different bacteria in blood group AB with and without AA. **(A)** *P. aeruginosa*, **(B)** *E. coli*, **(C)** *P. mirabilis*, and **(D)** *A. baumannii*.

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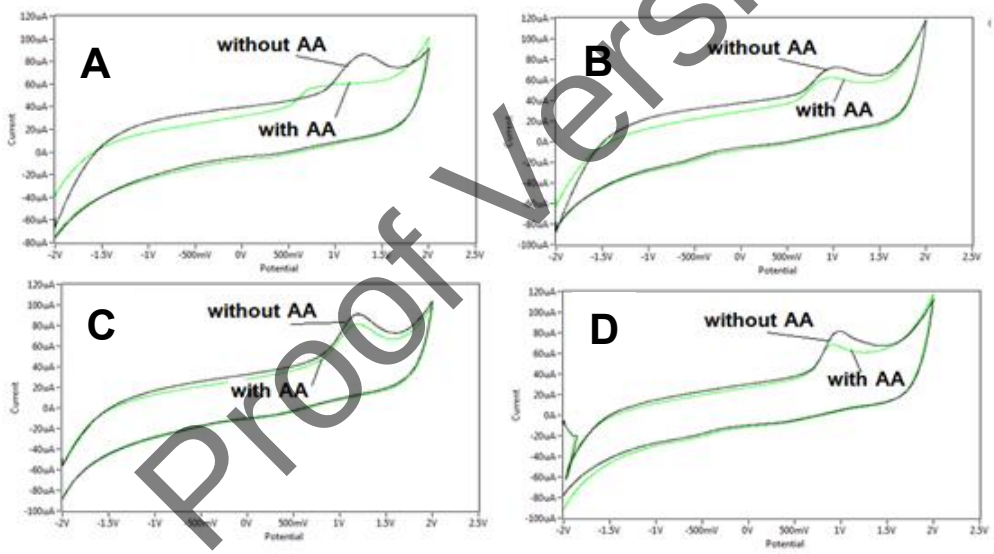


Figure 7. Cyclic voltammogram of different bacteria in ABO blood group O with and without AA. **(A)** *C. albicans*, **(B)** *E. coli*, **(C)** *P. mirabilis*, and **(D)** *P. aeruginosa*.

Effect of AA on the oxidation of ABO group AB inoculated with various microorganisms.

Culturing microorganisms under study in group AB plasma showed strong antioxidative results with AA, compared with its absence. As with the previous ABO blood groups, *A. baumannii* exhibited the highest effect (ΔIpa = 15), followed by *E. coli* (ΔIpa = 13), *P. mirabilis* (ΔIpa = 11), *C. albicans* (ΔIpa = 6), *S. aureus* (ΔIpa = 5), and *P. aeruginosa* (ΔIpa = 4), and the microorganism with the least effect was *K. pneumoniae* (ΔIpa=1) **(Figure 6)**.

Effect of AA on the oxidation of ABO Blood Group O inoculated with various microorganisms

Figure 7 illustrates the oxidative results when the microorganisms under study were cultured in group O plasma. A strong antioxidative effect was observed with AA compared with the condition without AA. Unlike the other ABO blood groups, the highest antioxidative potential was observed against *C. albicans* (ΔIpa = 31), followed by *S. aureus* (ΔIpa = 14), *E. coli* (ΔIpa = 13), *P. aeruginosa* (ΔIpa = 12), and *P. mirabilis* (ΔIpa = 11). In contrast, the least effect was observed against *A. baumannii* (ΔIpa = 3) and *K. pneumoniae* (ΔIpa = 2).

Discussion

As a pro-oxidant, antioxidant, free radical scavenger, and antibacterial molecule, vitamin C can alter the antimicrobial activity of different antibiotics and considerably reduce the harmful effects of ROS.[11] In addition to serving as a pro-oxidant antimicrobial molecule and a reducing agent that can quickly eliminate ROS, vitamin C may alter the antimicrobial efficacy of some antibiotics, potentially via two mechanisms: the first depends on the delivery of vitamin C into the bacterial cells, which leads to the release of H_2O_2 and, consequently, ROS. The second depends on the production of lactate and acetic acid from vitamin C. In addition, the combination of catalytically active metal ions with vitamin C may cause oxidative damage.[17]

Vitamin C inhibited bacterial growth at each tested concentration. However, the highest concentration (100 mg/mL) produced the largest IZ against gram-positive and gram-negative bacteria. Previous studies have reported similar findings.[18, 19] Higher concentrations of AA produced the largest IZs against gram-positive (e.g., *S. aureus*) and gram-negative (e.g., *E. coli*) bacteria.[18] AA in combination with antimicrobial agents demonstrated increasing antibacterial potential against various bacteria, including *E. coli, Salmonella, Yersinia enterocolitica, Listeria monocytogenes, S. aureus, Helicobacter pylori, P. aeruginosa*, and *Mycobacterium tuberculosis*.[20, 21] AA also inhibited the growth of certain *E. coli* strains. AA has considerable potential as an antibacterial agent, as demonstrated by Hassuna, *et al.* [17], in which high doses of AA decreased *E. coli* survivability. Furthermore, AA disrupts bacterial membrane integrity and inhibits metabolic processes [22, 23], thereby reducing their viability and proliferation. AA may have a critical function in mitigating the damaging effects of ROS generated during infections. In addition to its antioxidant capacity to fight infection, AA influences the immune system by enhancing immune cell function, eliminating microbes, and reducing inflammation. [14,24] Other functions include improved phagocyte function, T-lymphocyte maturation, interferon production, and disruption of viral replication.[12] AA has been proposed as a safe and effective antimicrobial agent that works independently against gram-positive and gram-negative bacterial strains, including resistant strains, while remaining stable at different temperatures.[18,19] A previous study also showed that AA has a substantial independent antibiotic effect (under *in vitro* and *in vivo* settings) in a mouse model against carbapenem-resistant hypervirulent *K. pneumoniae*.[25]

Overall, this study demonstrated the effectiveness of AA as an antioxidant agent against different bacteria (*S. aureus*, *P. mirabilis*, *E. coli*, *K. pneumoniae, A. baumannii,* and *P. aeruginosa*) and fungi (*C. albicans*) in different blood ABO groups.

Conclusion

The study found that AA inhibits the growth and activity of various pathogenic microorganisms when added to infected blood samples. This effect was observed in blood samples infected with bacteria such as *A. baumannii* and *P. mirabilis*, as well as the fungus *C. albicans*.

Author contributions

AK, RH, and EM contributed to the study's conceptualization and design. AK and EM conducted the experiments. AK and RH analyzed the data. AK and RH conducted the literature search. AK wrote the first draft of the manuscript. RH and EM edited the subsequent versions. All authors approved the final version submitted for publication and take responsibility for statements made in the published article.

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Conflict of interest statement

None to declare.

Data sharing statement

All generated data are included in the study.

AI declaration

No AI tools were used in the study.

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