Supplementary Information for

Antimicrobial silver: An unprecedented anion effect

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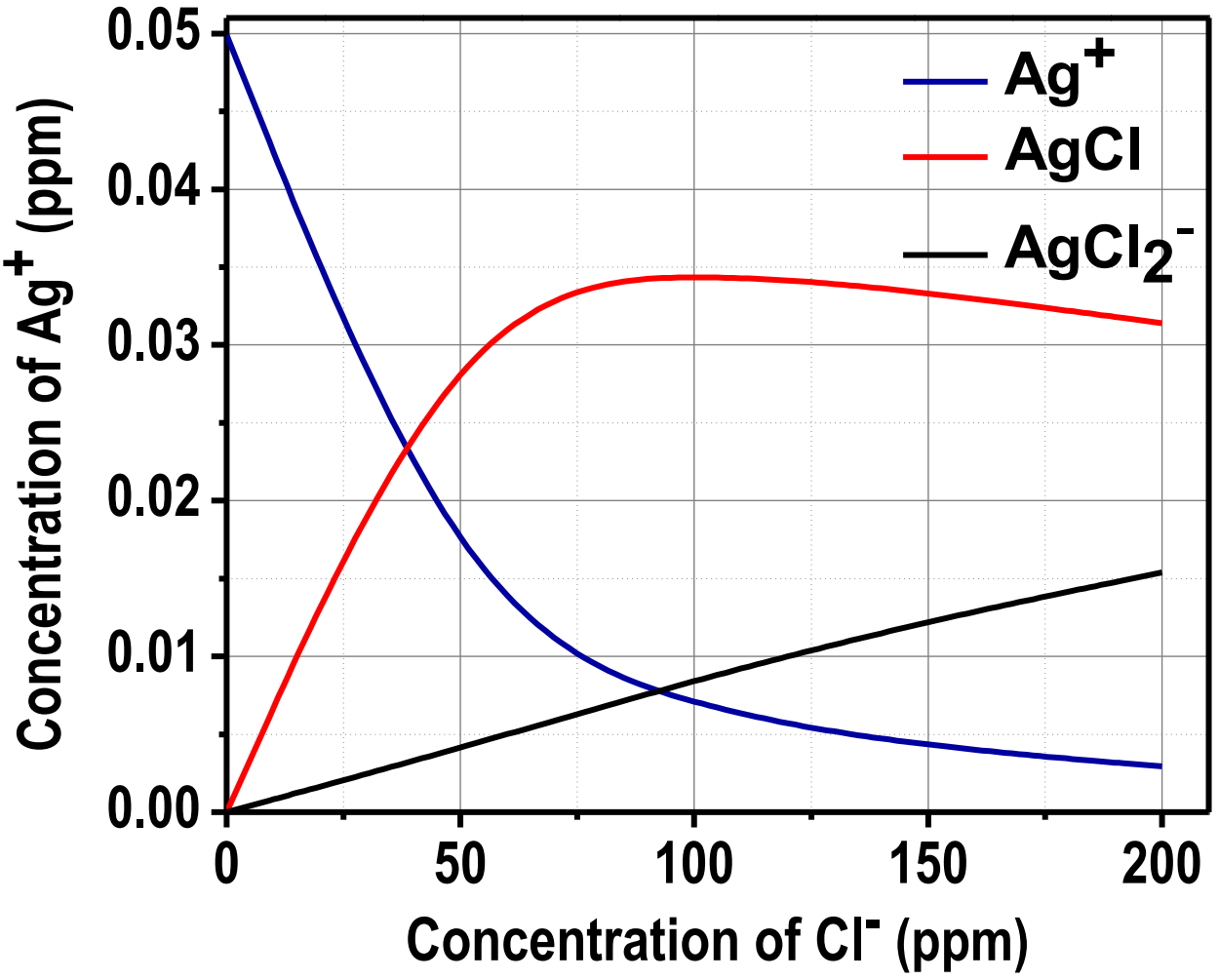
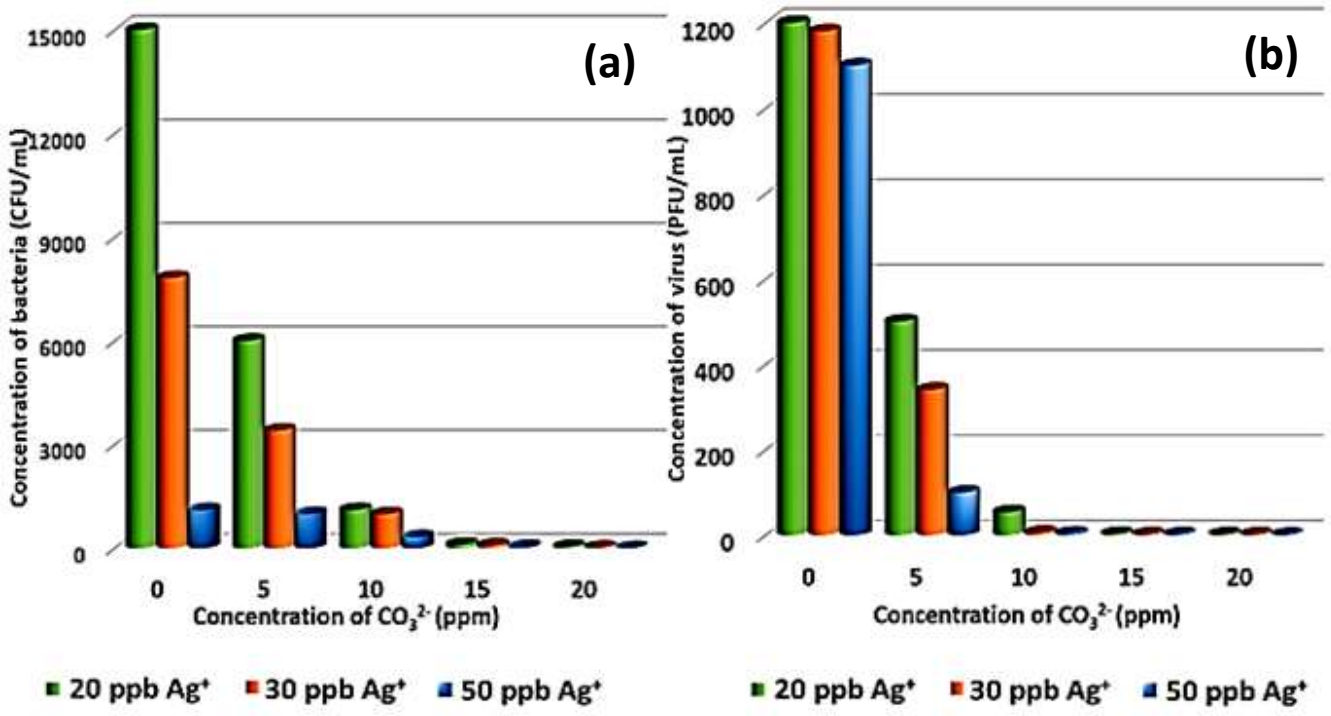


Supplementary Figure S1

Speciation diagram of silver ion in pure water: The speciation diagram prepared by varying [Cl^-] and keeping [Ag_{total}] = 50 ppb, pH = 7, temperature = 25°C. The graph shows the number of complexes formed due to speciation of 50 ppb Ag^+ at different chloride ion concentration. This speciation graph infers that higher the Cl^- concentration, lower is the Ag^+ concentration. The speciation diagram is prepared using simulations run on Visual MINTEQ software version 3.1 (freeware, available at, http://vminteq.lwr.kth.se).

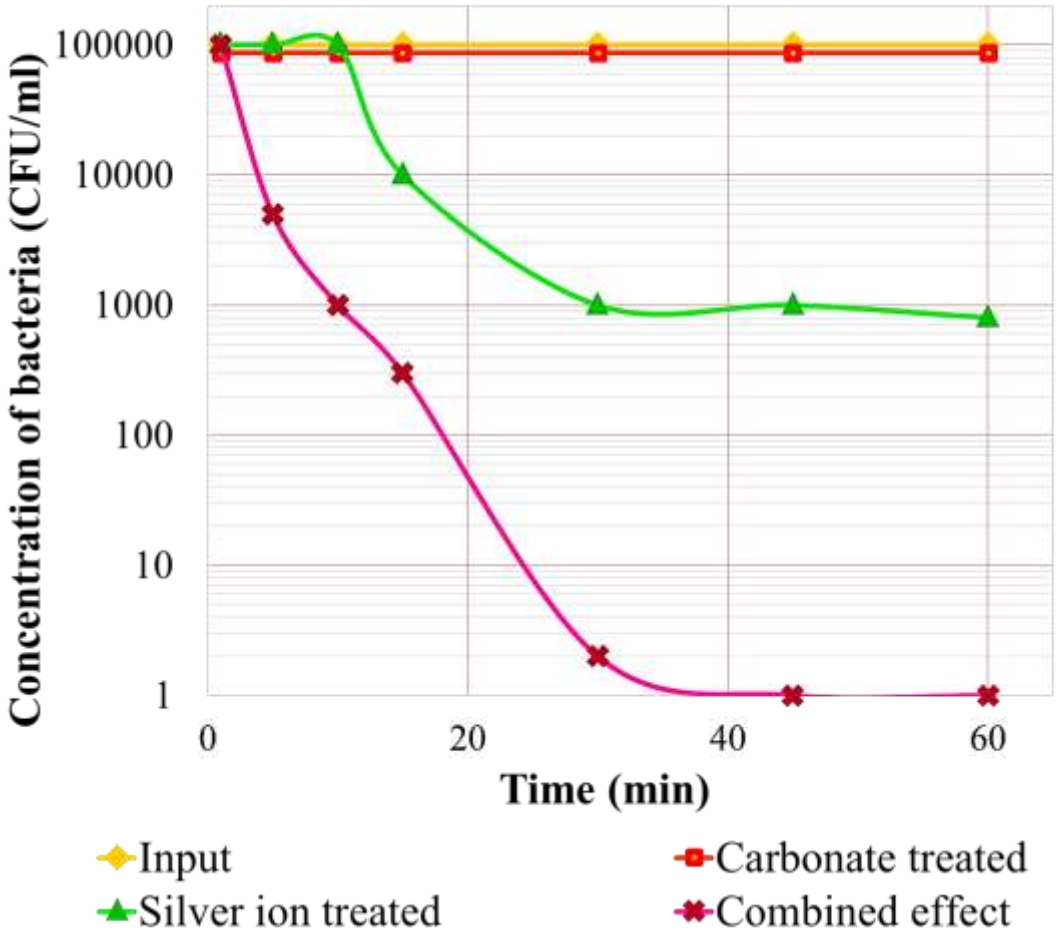
Supplementary Figure S2

Stability of silver ions in the test water: Concentration of silver ions in ppb available in solution as a function of time. Experiments have been done to assess the concentration after centrifugation to ensure that the estimation is for the species in solution.



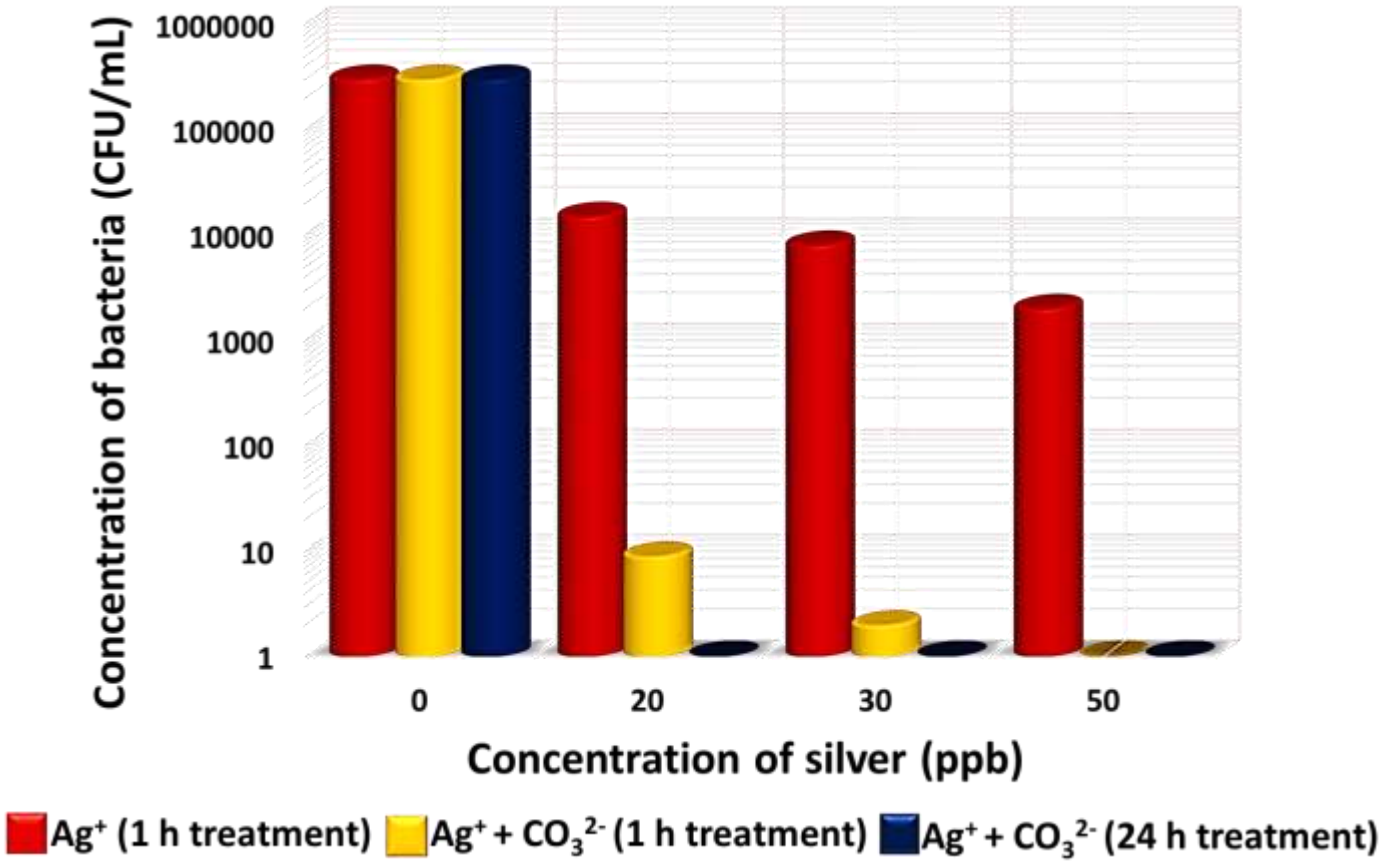
Supplementary Figure S3

Carbonate-supported antimicrobial activity of silver [A] Bacteria: *Escherichia coli* (ATCC 10536) [B] Virus: F-specific bacteriophage MS2 (ATCC 15597-B1) on *E. coli* host C-3000 (ATCC 15597): (A) Graph represents the optimization study of silver concentration on bacteria in which Ag^+ at 50 ppb reduced the input concentration (10^5 CFU/mL) by 2 log whereas in the presence of 20 ppm of CO_3^{2-}, Ag^+ at 20 ppb reduced the viable bacterial count to zero. (B) Graph represents the optimization study of silver concentration on virus in which Ag^+ at 50 ppb did not reduce the input concentration (10^3 PFU/mL), whereas in the presence of 20 ppm or less of CO_3^{2-}, 20 ppb Ag^+ reduced the viable viral count to zero.



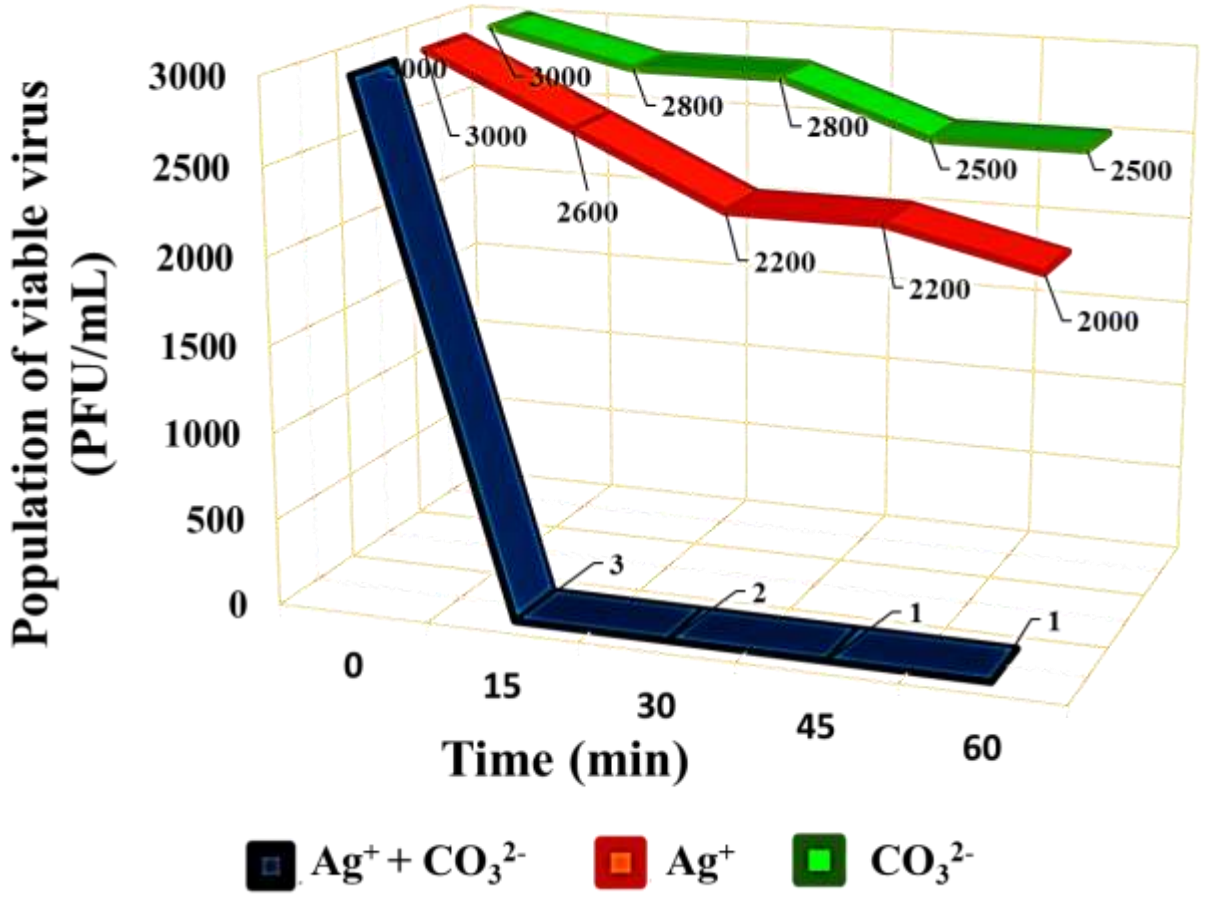
Supplementary Figure S4

Rate of antibacterial efficiency of Ag^+ in the absence and presence of CO_3^{2-}: Enhancement in the rate of antimicrobial property obtained by $Ag^+ + CO_3^{2-}$. In experimental water, Ag^+ showed 100 times reduction, CO_3^{2-} individually showed no significant antimicrobial property, whereas their combination was 100% effective. This reduction was obtained within a contact time of 15 minutes in the case of $Ag^+ + CO_3^{2-}$, but was not observed by Ag^+ treatment even after a longer contact time.



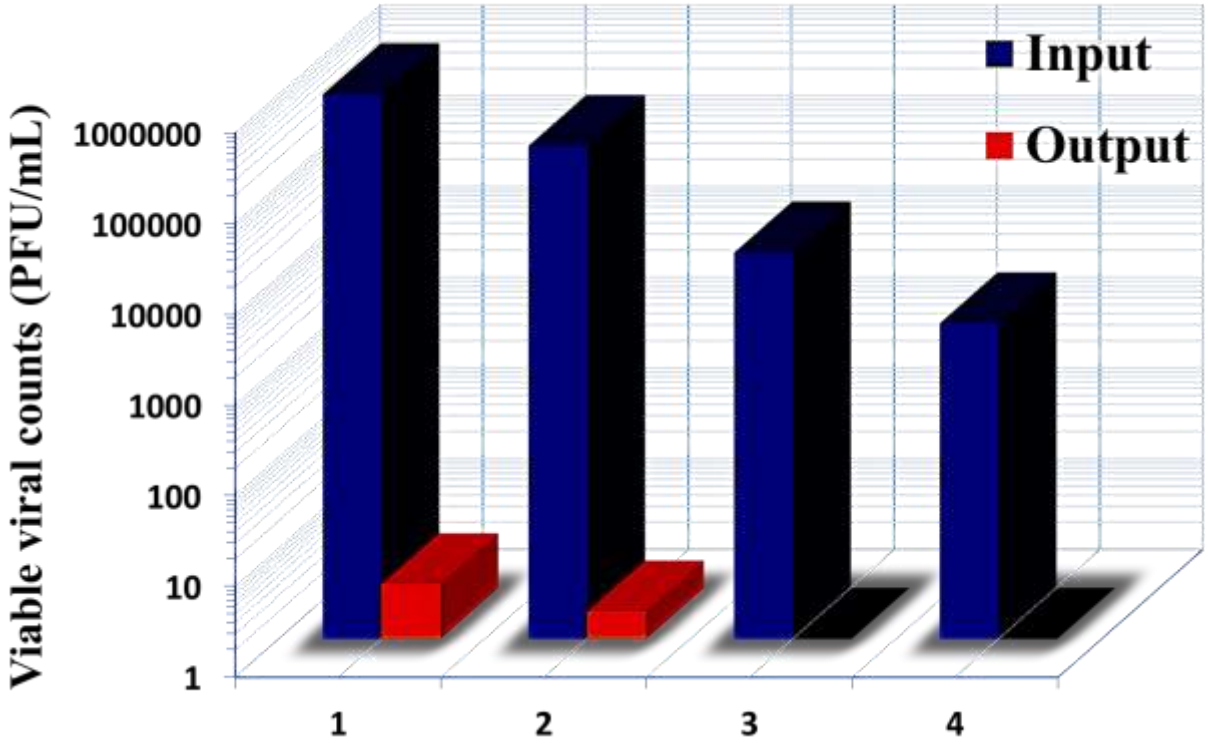
Supplementary Figure S5

Comparison of the antibacterial activity of Ag^+ and $Ag^+ + CO_3^{2-}$ against gram positive bacteria, *S. aureus* (ATCC 9144): Graph represents the comparison between the antimicrobial effects obtained by Ag^+ and CO_3^{2-} individually and in combination. While CO_3^{2-} individually showed no antimicrobial activity against gram positive *S. aureus*, Ag^+ showed a 3 log reduction and the combination showed a 5 log reduction of the input bacteria (10^5 CFU/mL) after a contact time of 1 h. On 24 h standing time, the results show that this activity was bactericidal and was not bacteriostatic.



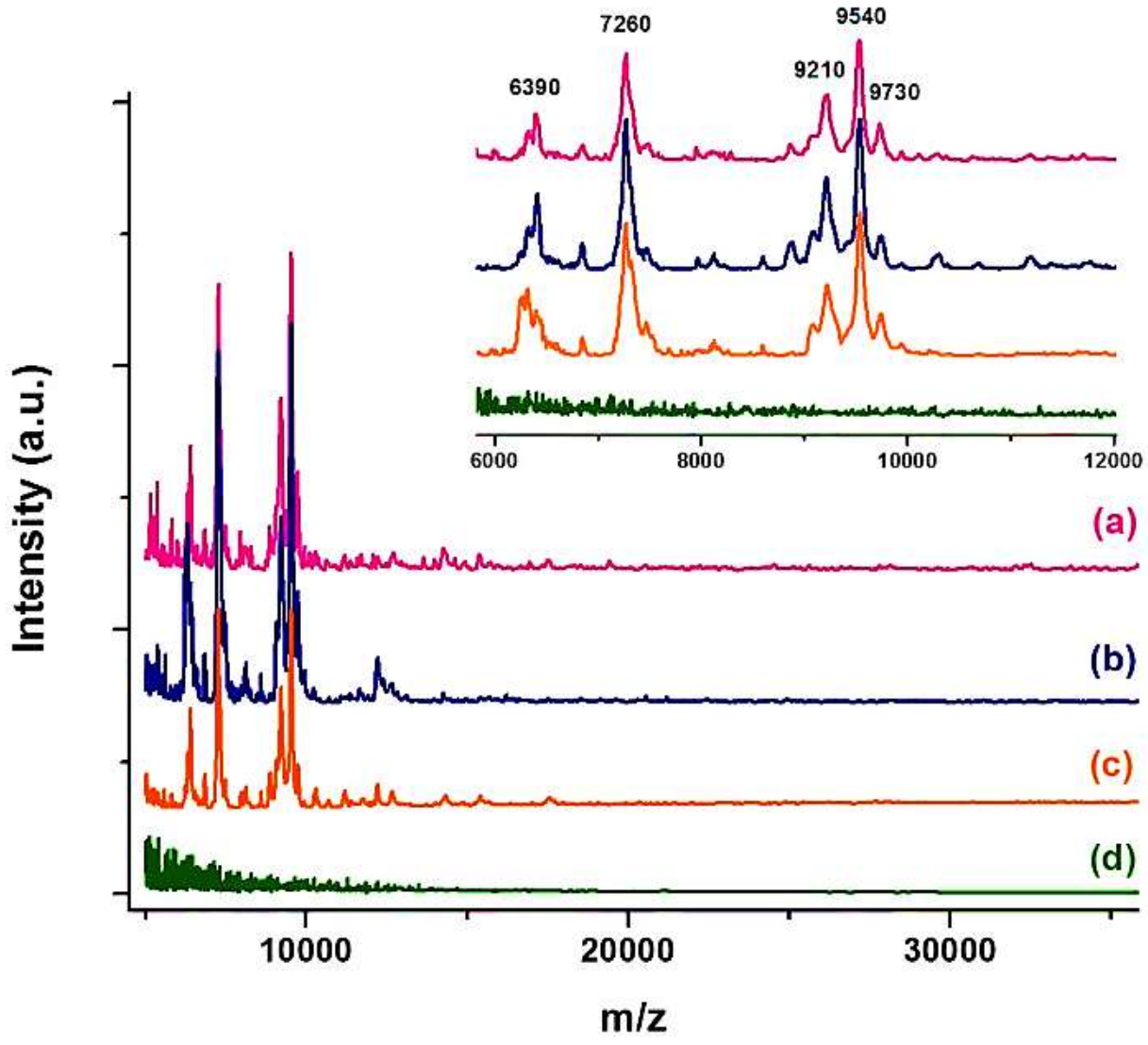
Supplementary Figure S6

Rate of virus killing efficiency for Ag^+ in the absence and presence of CO_3^{2-}: Graph represents the enhancement in the rate of antiviral property obtained by Ag^++ CO_3^{2-}. Neither Ag^+ nor CO_3^{2-} individually showed a significant antiviral property for MS2 bacteriophage while their combination was 100% effective. This reduction was obtained within a contact time of 15 minutes in the case of Ag^++CO_3^{2-}, but was not observed by Ag^+ treatment even after a longer contact time.



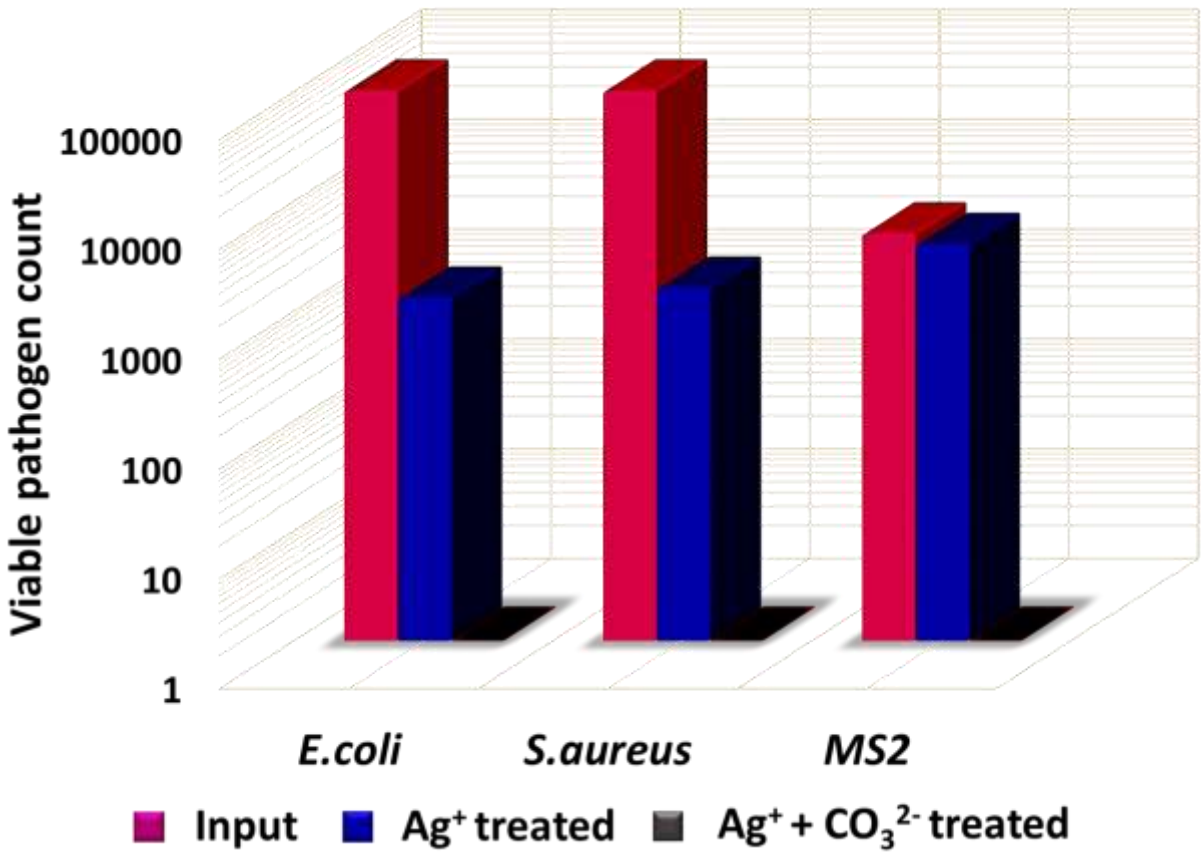
Supplementary Figure S7

Efficiency of Ag^{+}+ CO_3^{2-} on higher concentration of viruses: Graph represents the antiviral property obtained by Ag^{+} + CO_3^{2-} against higher viral load: Input concentrations of (1) 10^6, (2) 10^5, (3) 10^4 and (4) 10^3. The output virus concentration after treatment with [Ag^{+}] = 50 ppb and [CO_3^{2-}] = 20 ppm. Studies were conducted in synthetic challenge water for a contact time of 1 h**.**



Supplementary Figure S8

Mass spectra of the peripheral membrane proteins/peptides using MALDI TOF MS: Mass spectra of the peripheral membrane proteins/peptides using MALDI TOF MS (a) Control – The bacteria without any treatment, (b) 50 ppb Ag^{+} treatment, (c) 100 ppm Ag^{+} treatment, (d) Bacteria after CO_3^{2-} treatment. The CO_3^{2-} treatment should have removed the peripheral proteins/peptides on the membrane which had eluted away with the supernatant and thus showed no peak when the peripheral protein-free cells, subjected to sonication were measured under MS. Inset shows MALDI TOF spectrum of region m/z 6,000 to 12,000.



Supplementary Figure S9

A quantitative expression of enhanced antimicrobial activity observed in the presence and absence of CO_3^{2-}: A quantitative expression of the enhanced antimicrobial activity observed in the presence and absence of CO_3^{2-}. A 5 log reduction in the case of bacteria and a 3 log reduction in the case of virus were observed on 25 ppb Ag^+ + 20 ppm CO_3^{2-} treatment. Bacterial counts in most of the measurements were zero for 25 ppb Ag^+ + 20 ppm CO_3^{2-} treatment, but in a few cases counts of 1 or 2 were also seen in 1 h treatment which went to zero in 24 h treatment.

Supplementary Table S1

Physicochemical characteristics of influent natural water:

(Note: All parameters are expressed in mg L^{-1}, except for pH and conductivity)

Parameters	Value
Total coliforms (CFU/mL)	1-2 x 10^3
p H @25°C	7.8
Conductivity (µS/cm)	640.000
Fluoride	0.573
Chloride	86.340
Nitrate	1.837
Sulphate	32.410
Silicate	15.870
Lithium	ND
Sodium	53.740
Ammonium	ND
Potassium	2.330
Magnesium	14.340
Calcium	28.720

ND-not detected

Supplementary Note 1

MALDI MS – Sample Preparation:

The peripheral membrane proteins that are suspected to be affected by the carbonate treatment were separated from the cells and analyzed under mass spectrometry (MALDI TOF MS). Here, the cells were initially treated with 50 ppb Ag^{+}, 20 ppm CO_3^{2-} and 100 ppm Ag^{+}. After treatment, the cells were separated from the solution and were subjected to sonication for the removal of peripheral proteins/peptides. In the case of control and Ag^{+} treatment, the peripheral proteins/peptides were not affected during the reaction time. Thus, these cells on sonication, released low molecular weight proteins into the solution and were detected by MALDI MS. Whereas CO_3^{2-} treatment removed the proteins and the cells when separated and processed did not contain these proteins to be detected by MALDI MS.

For MALDI TOF MS analysis, an Applied Biosystems Voyager De Pro instrument was used with sinapic acid as the matrix. A pulsed nitrogen laser of 337 nm was used for ionizing the sample. Spectra were collected in the negative mode and an average of 250 shots were used for each spectrum. The matrix was prepared by dissolving 10 mg of sinapic acid in a 1:3 mixture of acetonitrile: 0.1% trifluoroacetic acid (overall volume of 1 mL). While preparing samples for analysis, 5 µL of the supernatant solution, without dilution, was mixed thoroughly with 100 µL of the matrix mixture. 2.5 µL of the resulting mixture was used for spotting.