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Research Article

Use of Negative and Positive Ions for Reducing Bacterial Pathogens to Control Infections

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Abstract

In recent years, there has been renewed interest in the use of air ionizers to control the spread of airborne infections. Bacteria and viruses attached to air particles circling in a room can be charged by ions, so they fall out and are removed from the air. Furthermore, negative and positive ions have been shown to have microbicidal effects on various bacterial and fungal species. Thereby the use of ionizers may contribute to disinfecting the atmosphere and stopping the transmission of microorganisms. The aim of this study was to determine if the sensitivity of bacteria to ions could be influenced by variation in experimental parameters.

Keywords: Negative; Positive; Reducing Bacterial

Introduction

Ionized air molecules make up more than a very small percentage of the atmosphere but nevertheless they play a profound role in maintaining the health of the atmosphere by removing the particulate and the chemical pollutants. The natural sources of ionization include the effects of radiation, effects of the Earth's electric field, the movements of wind and the splashing of water (Lenard Effect) [1]. The life of negative (NAIs) and positive (PAIs) air ions is approximately 100 seconds [2], and it is affected by humidity, temperature and oxygen concentration. Primary species of NAIs are superoxide ion (95%), O^-, OH^- and O_2^- instead CO_3^-, O_2^- (H_2O) and OH^- (H_2O) constitute secondary NAIs. As for the PAIs, the main species produced are O_2^+, O^+, N^+ and H_3O^+ [3]. An excess of negative ions is considered as beneficial on health and air quality, while positive ions are said to possess unpleasant properties. Artificial generator of small ions is relatively simple. Most of the so-called ionizers are based on the principle of corona discharge which mainly produce negative ions, and the Plasma Cluster ionizers, which produce positive and negative ion clusters and are, among the models in use, the most recent nowadays [4]. Hence, in recent years there has been renewed interest in the use of air ionizers to control the spread of airborne infections [5]. The ionizers, in fact, produce air ions that collide with suspended particles and give them a charge; charged particles aggregate together and fall out of the air. Even bacteria and viruses circling in a room can be cleared by negatively charged ions attaching to them and removing them from the air [6]. Over many years, various researchers have reported that air ions have a biocidal action because inhibit the growth of various bacterial and fungal species due to the oxidative stress they generate [5,7]. There are several suggested mechanisms for the action of the ionizers: electrical phenomena (ions, electric charge, ozone production) and electrostatic repulsion [7]. Thereby the application of ionizers may have the potential contribute to disinfecting the atmosphere such as the domestic environment [8], in food safety [9], for reduction in airborne and surface bacteria inside the cars' cabins [10] and stopping the transmission of infections such as some hospital acquired infections (HAIs) [5,11].

The aim of this study is to determine if the sensitivity of Gram-positive and Gram-negative bacteria to ions could be influenced by variation in experimental parameters

Methods

Generating air ions

Plasma Cluster ionizers were supplied by an Italian company. Through plasma discharge, in which voltage is applied to the discharge electrode, positive hydrogen ions (H^+) and negative oxygen ions (O_2^-) are generated from the water and oxygen in the air releasing balanced shower of positive and negative ions. The Plasma Cluster ions have an agglutinating property to the surface of fine particles, thereby surrounding harmful substances such as airborne mould spores, viruses and allergens. Then, they initiate a chemical reaction and change into highly reactive hydroxyl radicals (OH), which rob hydrogen (H) from the harmful substances to break them down. The robbed hydrogen combines with the hydroxyl radicals to form water molecules and returns to the air (Figure 1).

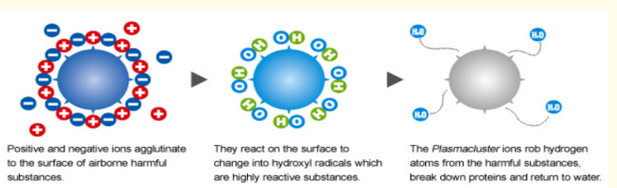


Figure 1: PlusmaCluster technology

We used this technology to determine whether its effect on bacterial cells depends on: i) the bacterial type; ii) the bacterial load; iii) the action area and iv) the distance of the ion generator

Ion capture

To measure NAI and PAI emitted concentration, we used an Air Ion Counter. It is a handheld meter designed to measure ion density – the number of ions per cubic centimeter (ions/cm3) – in air. It can measure this number separately for positive and negative ions (these ions are usually present simultaneously in the air). It contains a fan which pulls air through the meter at a calibrated rate. Air is sucked in at the top of this instrument, measured and ejected at the bottom. The display shows the ion count and it continues to display the ion density in the air, showing any changes.

Microbiological assay

Bacterial strains and culture conditions We used the strains *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. Cultures were grown on Nutrient Broth at 37 °C overnight in an incubator.

Exposure to negative and positive ions

Ion exposure experiments were undertaken in aerobic sterile conditions at room temperature and 40 - 60% relative humidity (RH) into a laminar flow cabinet. Temperature and RH were constantly monitored with Humidity/Temp Data Recorder. Bacterial cultures were serially diluted in 0.9% NaCl saline solution to obtain two different inoculum concentrations: 104 and 107 UFC/ml respectively. Each dilution was spread on Nutrient Agar medium using Petri-dishes of two different diameters (Ø 90 mm or 150 mm). Then bacteria suspension plated on Petri dishes, without lids, were placed under the ionizer, at the distance of 5 cm or 10 cm from the ion source and exposed to the negative and positive ions for period of 1, 3, 8 and 12 hours, with their relative growth control. After the exposure all Petri-dishes were incubated at 37 °C for 18-24 h and the number of CFU/ml was counted. The air ion exposure experiments were repeated in twice.

Statistical analysis

Data from the *S. aureus* ATCC 29213 and E. coli ATCC 25922 test at concentration of 104 UFC/ml were expressed as a percentage variation with respect to the own growth controls with the following formula:

$$\%\ Vitality\ Reduction = \frac{X_f - X_i}{X_i} \cdot 100$$

where:

X_f indicates the number of colonies after ionic treatment;

To compare the data obtained between the two microorganisms, the distance at which the ionizer is placed and the effect over time, the T-Student Test was used. The comparison was considered statistically significant for $p < 0.05$.

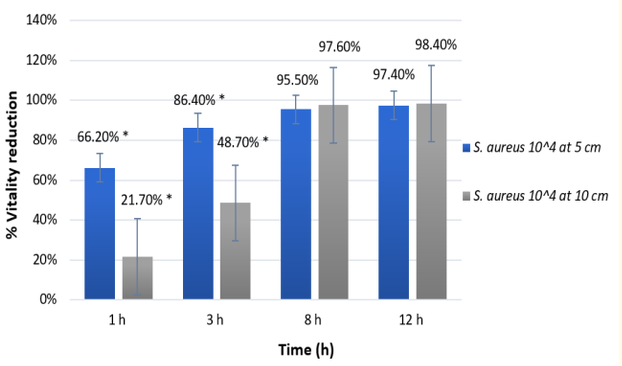
It was not possible to obtain a precise vitality reduction percentage for the inoculum of 10^7 CFU/ml, because the bacterial patina grown on the control plate did not allow the colony counting.

Results

The Air Ion Counter revealed that the average concentration of positive and negative ions emitted by the ionizer was 12 million ions/cm^3 at 5 cm of distance and 2.6 million ions/cm^3 at 10 cm of distance (Table 1). The microbiological results evidenced a promising efficacy of ions on both the Gram positive or negative bacterium, used at both concentrations (10^4 or 10^7 CFU/ml), plated on Petri-dishes of both diameters (90 or 150 mm) at both distances (5 or 10 cm) from the ionizer. Statistically significant differences in vitality reduction percentage for bacteria at 10^4 CFU/ml, plated on 150 mm Petri-dishes, with the ionizer placed at 5 or 10 cm of distance, are shown in Graphics 1-3. In particular, the difference in the percentage of bacterial vitality reduction, observed between the two distances, is significant ($p < 0.05$) only in the first hours of exposure, decreasing in the subsequent ones, almost disappearing after 12 hours (Graphics 1,2), underlying an interesting ion effect also when the ion source is at a greater distance Furthermore, the data related to the 7 4.

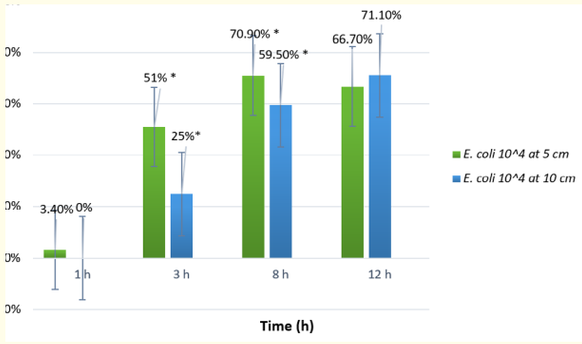
		Plasma Cluster Ionizator 1		Plasma Cluster Ionizator 2	
		Distance from Petri dishes		Distance from Petri dishes	
		5 cm	10 cm	5 cm	10 cm
Neutral position	Positive ions [million ions/cm^3]	11.3	2.8	13.3	3.1
	Negative ions [million ions/cm^3]	12.7	2.3	10.7	1.9

Table 1

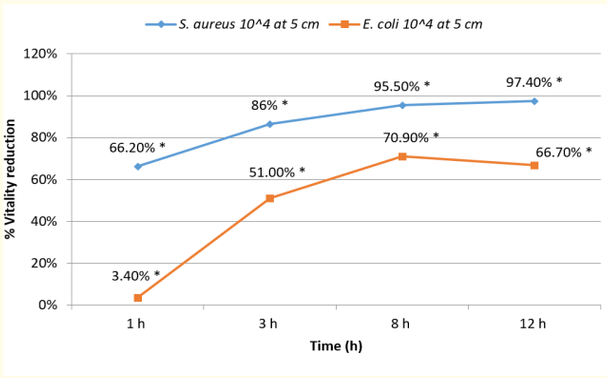


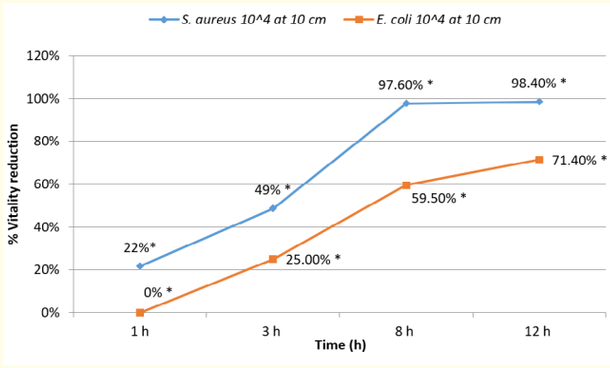
Graphic 1: Ion effects on *S. aureus* at 104 CFU/ml on 150 mm Petri dishes, with the ionizer placed at a distance of 5 and 10 cm.

* p<0.05 significantly different from control.



Graphic 2: Ion effects on *E. coli* at 104 CFU/ml on 150 mm Petri dishes, with the ionizer placed at a distance of 5 and 10 cm. * p<0.05 significantly different from control.





Graphic 3: A. Comparison of ion effects between *S. aureus* and *E. coli* at 104 CFU/ml on 150 mm Petri dishes, with the ionizer placed at a distance of 5 cm.

B. Comparison of ion effects between *S. aureus* and *E. coli* at 104 CFU/ml on 150 mm Petri dishes, with the ionizer placed at a distance of 10 cm.

Plasma Cluster ionizers were supplied by an Italian company. Through plasma discharge, in which voltage is applied to the discharge electrode, positive hydrogen ions (H^+) and negative oxygen ions (O_2^-) are generated from the water and oxygen in the air releasing balanced shower of positive and negative ions. The Plasma Cluster ions have an agglutinating property to the surface of fine particles, thereby surrounding harmful substances such as airborne mould spores, viruses and allergens. Then, they initiate a chemical reaction and change into highly reactive hydroxyl radicals (OH), which rob hydrogen (H) from the harmful substances to break them down. The robbed hydrogen combines with the hydroxyl radicals to form water molecules and returns to the air (Figure 1).

comparison between ion effects on the two bacterial strains at 104 CFU/ml show, in all the experimental conditions, an interesting biocidal activity (Graphic 3).

In detail, the vitality reduction is always higher for *S. aureus* than *E. coli,* confirming a better, and statistically significant for $p < 0.01$, ion antimicrobial activity against the Gram positive bacterium placed at a distance of 5 cm (Graphic 3 A) and 10 cm (Graphic 3 B) from the ionizer, respectively. Representative images that evidenced a more pronounced and significant ($p < 0.05$) ion activity observed on *S. aureus* when it was used both at 104 or 107 UFC/ml, independently from the distance from the ionizer and from the plate diameter, already starting from 3 hours of incubation and up to 12 hours are shown in figures 2 and 3.

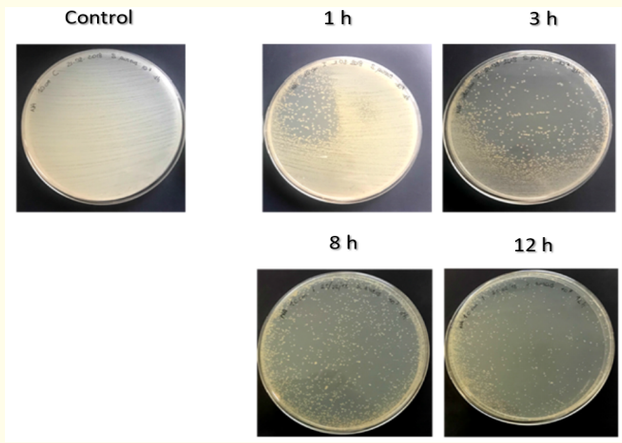


Figure 2: Ion effects on *S. aureus* plated at 107 CFU/ml on 150 mm Petri dishes, with the ionizer placed at a distance of 10 cm.

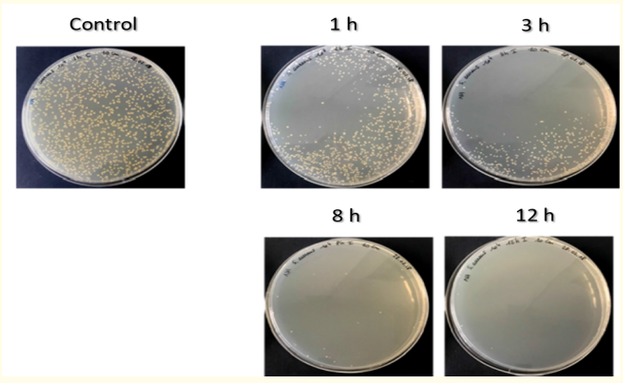


Figure 3: Ion effects on *S. aureus* plated at 104 CFU/ml on 150 mm Petri dishes, with the ionizer placed at a distance of 10 cm.

Conclusion

These results show how the antimicrobial effects of the ions depend a lot on bacterial strain features, on its concentration and, to a lesser extent, on the distance at which the electrodes are placed. About the bacterial type, the difference between the Gram positive and the Gram negative bacteria could be due to both a different composition of the cell wall or a greater antioxidant activity exerted by E. coli. Therefore, the study underlines how the development of new technologies such as ionizers can became promising alternative treatment for the decrease of microbial load in various field, such as healthcare facilities, with the potential to reduce the amount of antimicrobials used.

Acknowledgment

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