

**DIPARTIMENTO DI MEDICINA ANIMALE, PRODUZIONI E SALUTE – MAPS**  
**DEPARTMENT OF ANIMAL MEDICINE, PRODUCTION AND HEALTH**

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Legnaro, Italy, October  
10<sup>th</sup> 2016

## **Assessment of bacteria removal after environmental ozone treatment, performed by Sany Water Plus device built by Sanity System S.r.l. Corporation.**

### **Introduction**

Ozone is an oxidative gas with disinfectant power stronger than chlorine's. The oxidation and peroxidation of bio-molecules due to ozone action, produce toxic effects on cells. Indeed, the reactive molecules of oxygen cause damages to cellular lipids, proteins and DNA.

Ozone, inducing a drastic reduction of quantity of microorganisms, could be used to disinfect water, air and environment.

### **Aim of the study**

The aim of the study was to assess the effectiveness of environment air ozonizing in the reducing the quantity of multidrug-resistant bacteria of animal origins.

### **Methods and Materials**

#### **Environment**

Tests were carried out in two parallel rooms (room 1 and room 2) with the same characteristic (form and dimension). The air recycling was stopped and human

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activities were not conducted during the test. The room surface area was 18 m<sup>2</sup> and 54 m<sup>3</sup>.

### **Measurement of ozone concentration**

The concentration of produced ozone in treated room was measured through a specific device “Eco Sensors Model A-21ZX” (Eco Sensor, USA).

### **Experimental Test**

In the two rooms were placed plates inoculated with identical dilutions of selected multidrug resistant bacteria to perform a total plate count (TPC). The room 1 was submitted to the ozone treatment, while the room 2 was kept as negative control. After treatment, plates were incubated at 37°C for 24 hours. The effectiveness of the ozone treatment was assessed evaluating the decreasing of bacteria concentration (Colony-Forming Units/milliliter- CFU/ml) between the plates submitted to treatment and control.

### **Microorganisms**

Some aliquots of *Staphylococcus pseudintermedius* (*S. pseudintermedius*), *Enterococcus faecium* (*E. faecium*), *Enterobacter cloacae* (*E. cloacae*) and *Escherichia coli* (*E. coli*) were prepared dissolving bacteria in PBS with 20% of glycerol and were stored at -20°C until use. To perform the test an aliquot of each strain was defrosted, the bacteria were washed with PBS and the microorganisms were suspended in the saline solution.

### **Total plate count**

The saline solutions with dissolved bacteria were used to make decimally serial dilutions plated on Nutrient Agar plates (OXOID) (100µl/inoculum).

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The dilutions were used to perform the Time 0 count, the Negative control count and the count after Ozone treatment.

All counts were carried out in double.

### **Time 0**

The plates were immediately incubated at 37°C for 24 hours.

### **Negative control**

The plates were exposed to the air for 90 minutes in the room 2 (18 m<sup>2</sup>).

After this period of exposition, the plates were incubated at 37°C for 24 hours.

### **Ozone treatment**

The plates were placed in the room 1 (18 m<sup>2</sup>) and the ozone treatment was carried out using the Sany Water Plus device, setting up with the number 2 environment program, corresponding to the 20 m<sup>2</sup> calibration.

The plates were exposed until conclusion of the program 2 cycle and for a supplementary time, for a total of exposition time of 90 minutes.

After the treatment, the plates were incubated at 37°C for 24 hours.

The negative control and ozone treatment tests were performed simultaneously to ensure the same temperature and humidity.

### **Expression of the results**

The results were expressed in CFU/ml. The bacterial removal rate was calculated considering the difference in % between the negative control count (100%) and the bacteria count after the ozone treatment.

Each bacterial strain was tested three times to assess the repeatability of the treatment.

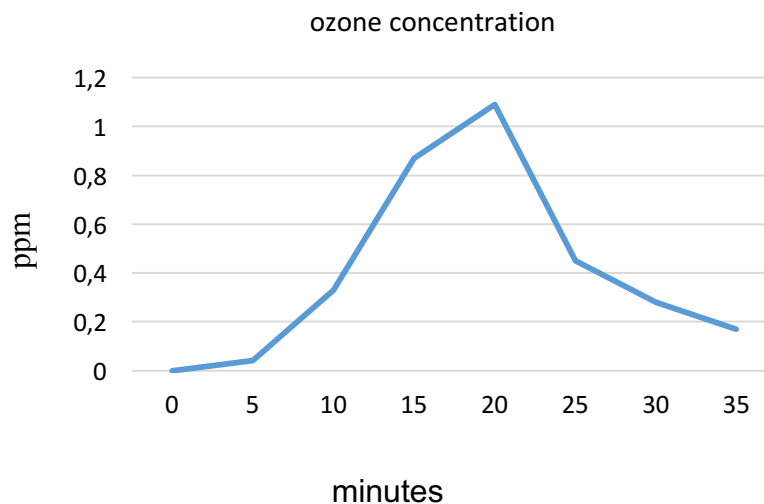
## Results

### Ozone production

The rooms designated to the experimental test had a surface area corresponding to 18 m<sup>2</sup> and 54 m<sup>3</sup>.

The room temperature was 24,7°C. After the start of the 2nd program of the Sany Water Plus device, the concentration of ozone was measured every 5 minutes. The treatment cycle lasted 35 minutes. The measurement of ozone allowed to detect a maximum concentration of 1,09 ppm. The peak of the concentration of ozone was reached after 20 minutes and it was kept for about 2 minutes (Figure 1).

**Figure 1**



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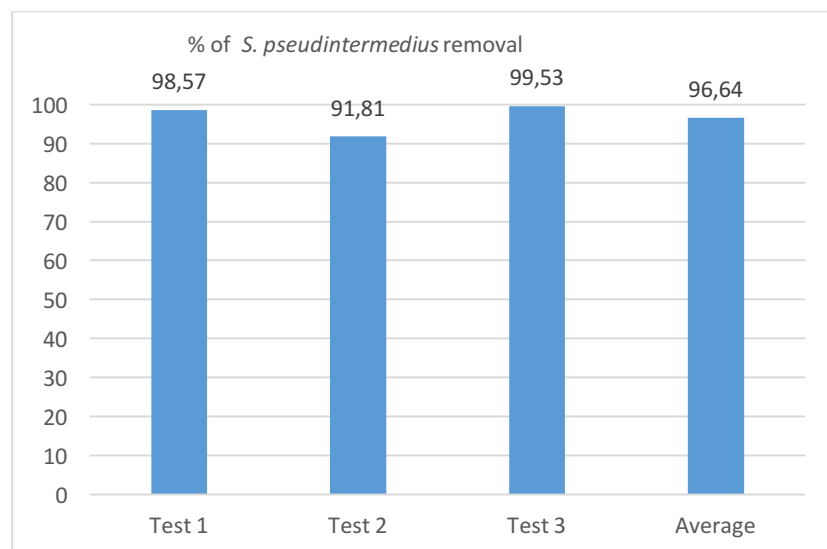
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## Outcomes of the experimental test

### *Multidrug resistant Staphylococcus pseudintermedius*

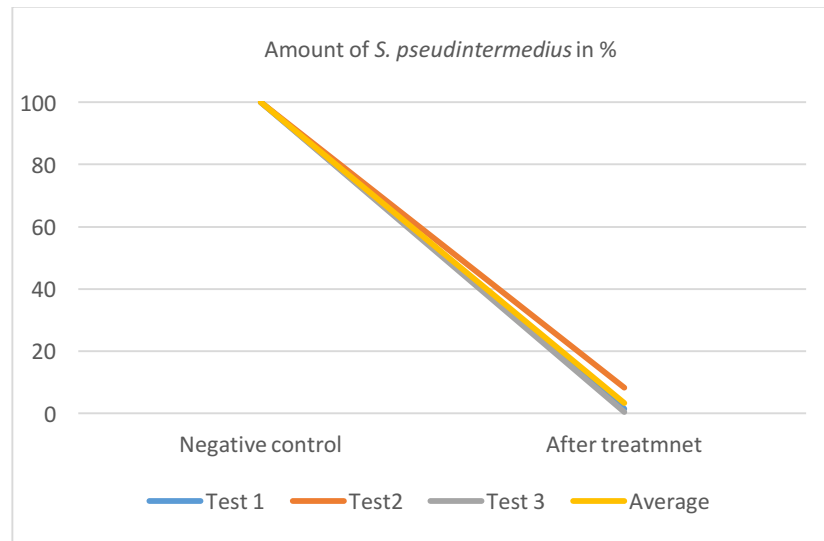
		Count at T0	Count of negative control	Count after ozone treatment	% of bacteria removal
<b>Test 1</b>	UFC/ml	1245	930	13,3	98,57
<b>Test 2</b>	UFC/ml	820	765	62,6	91,82
<b>Test 3</b>	UFC/ml	11900	11850	55,8	99,53

The removal % was calculated considering the difference between the negative control count (100%) and the count after ozone treatment.

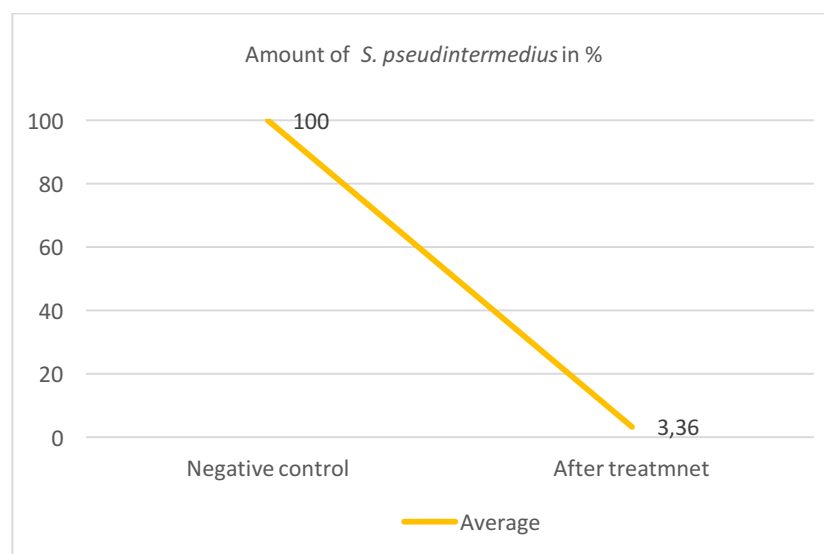


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The amount of *Staphylococcus pseudintermedius* after ozone treatment was 1,43% in the test 1, 8,19% in the test 2 and 0,47% in the test 3, with an average of 3,36%.



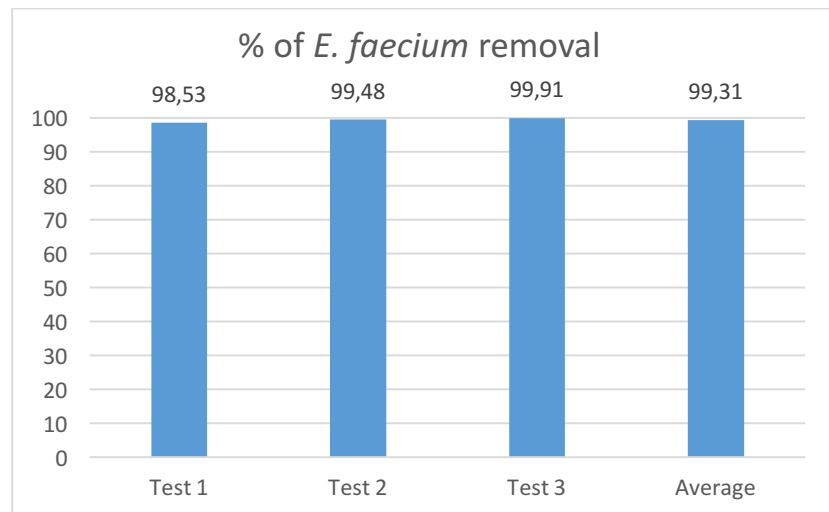
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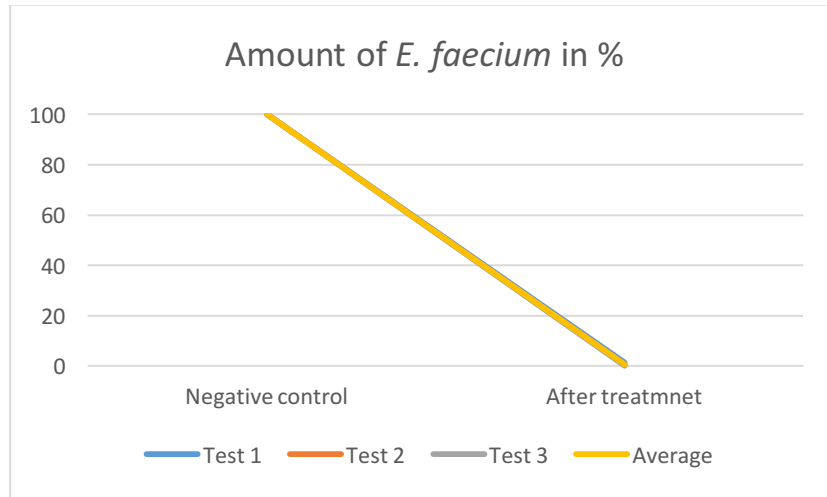
## Multidrug resistant *Enterococcus faecium*

		Count at T0	Count of negative control	Count after ozone treatment	% of bacteria removal
Test # 1	UFC/ml	4500	2730	40	98,53
Test # 2	UFC/ml	15000	12220	63	99,48
Test # 3	UFC/ml	13500	11050	10	99,91

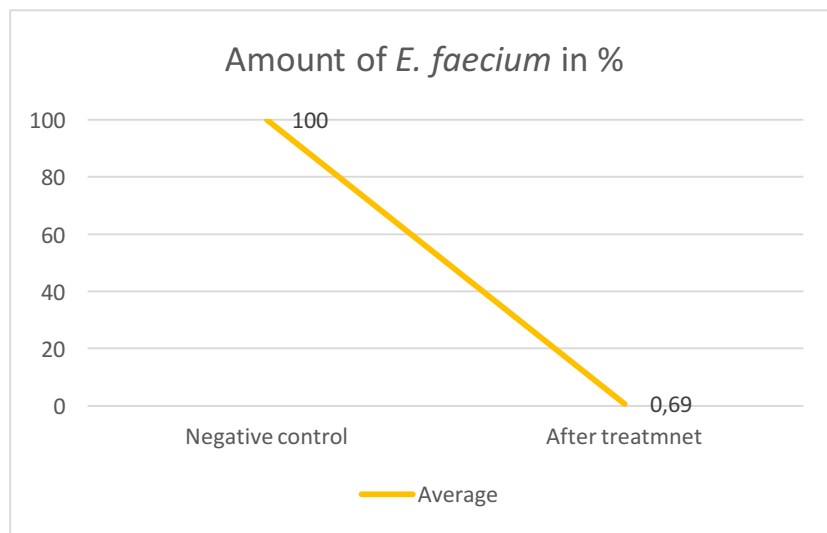


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The amount of *Enterococcus faecium* after the ozone treatment was 1,47% in the test 1, 0,52% in the test 2 and 0,09% in the test 3, with an average of 0,69%.





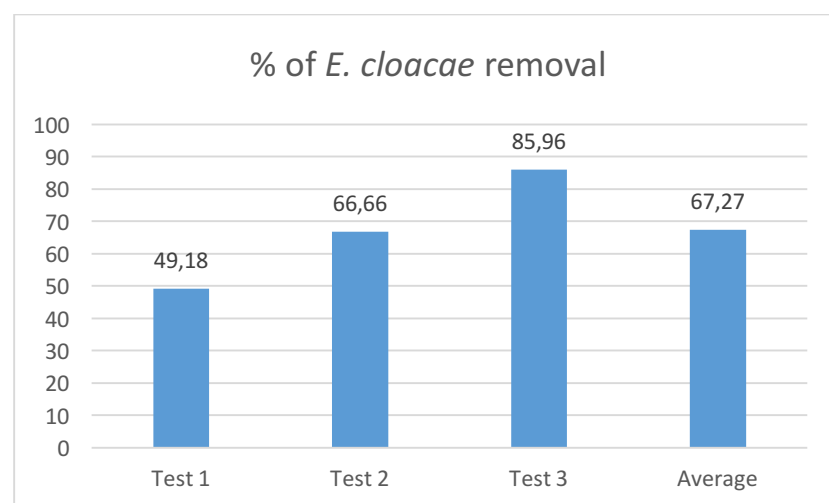
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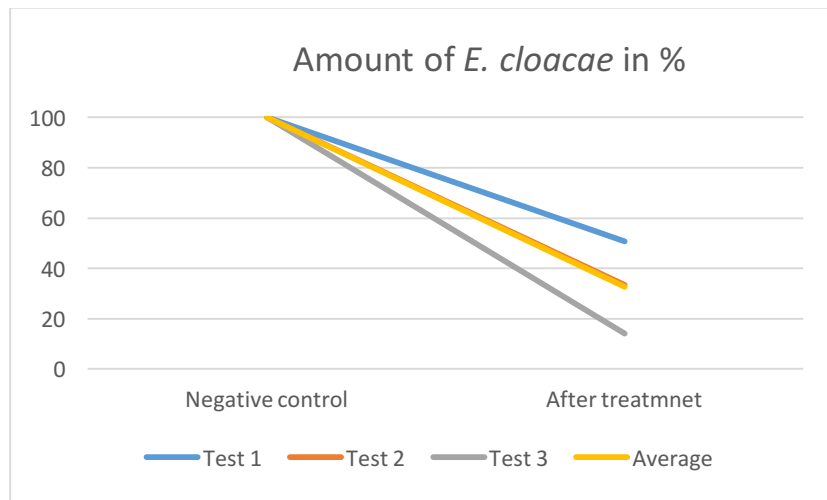
### Multidrug resistant *Enterobacter cloacae*

		Count at T0	Count at negative control	Count after ozone treatment	% of removal
	Unit of measurement				
Test # 1	UFC/ml	6150	6100	3100	49,18
Test #2	UFC/ml	6650	3375	1125	66,66
Test # 3	UFC/ml	84800	68400	9600	85,96

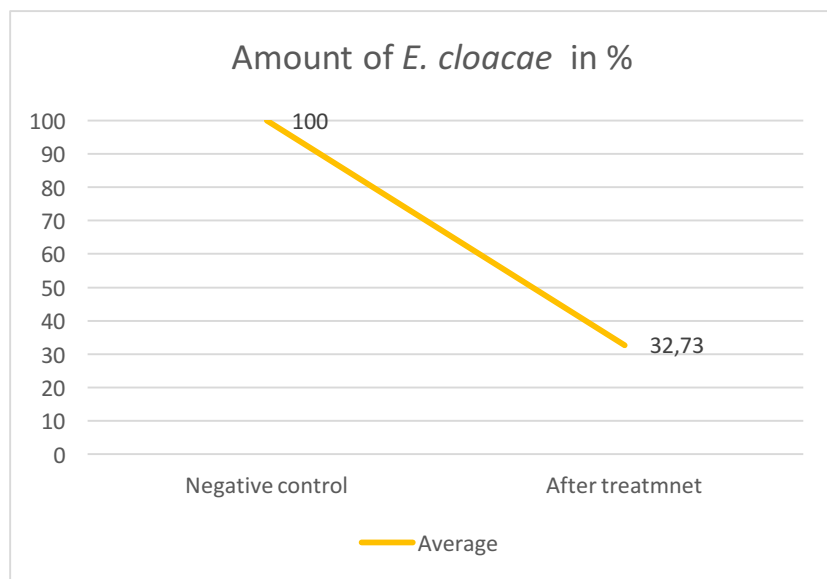


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The amount of *Enterobacter cloacae* after the ozone treatment was 50,82% in the test 1, 33,34% in the test 2 and of 14,04% in the test 3, with an average of 32,73%.

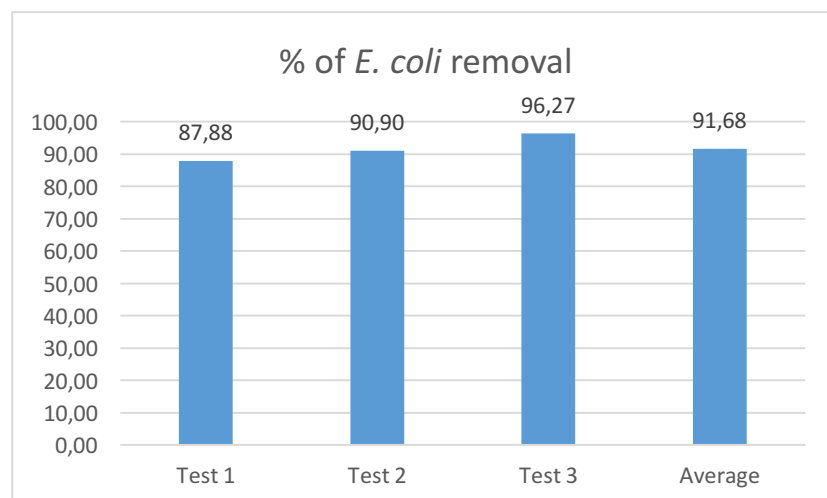


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### *Escherichia coli*

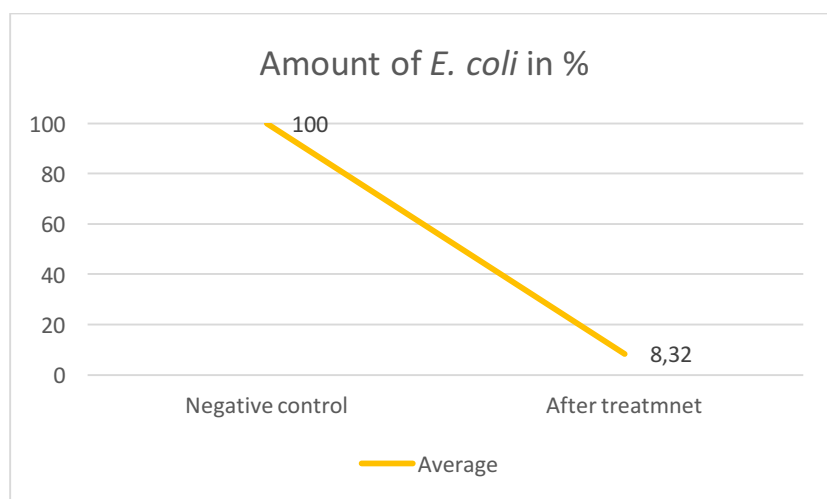
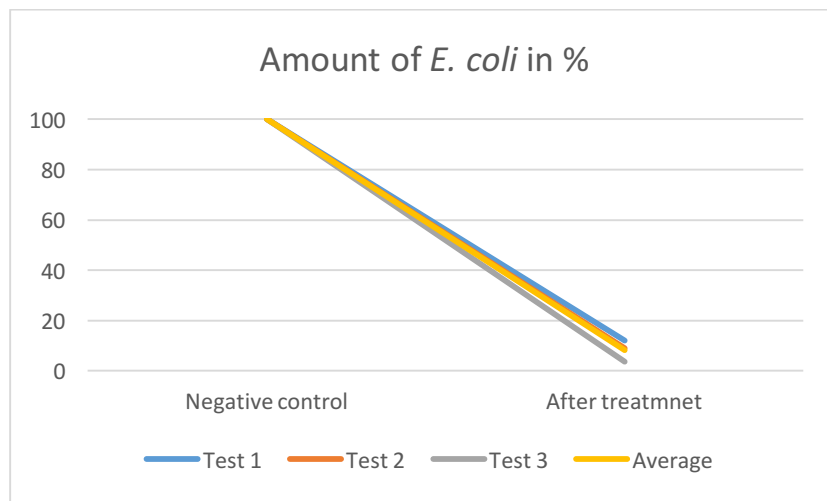
		Count at T0	Count at negative control	Count after ozone treatment	% of removal
	Unit of measurement				
<b>Test # 1</b>	UFC/ml	6260	6150	745	87,88
<b>Test # 2</b>	UFC/ml	7750	7750	705	90,90
<b>Test # 3</b>	UFC/ml	3508	3463	129	96,27



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The amount of *Escherichia coli* after the ozone treatment was 12,12% in the test 1, 9,1% in the test 2, and of 3,73% in the test 3, with an average of 8,32%.



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## Conclusions and discussion

The ozone causes toxic cellular effects due to irreversible damages of lipids and proteins of cellular components and leads to a drastic reduction of the microorganism.

The aim of this test was to verify the effectiveness of the ozone treatment produced by the Sany Water Plus device, against the microorganisms with the particular properties of resistance and pathogenicity .

These microorganisms represent a selection of bacteria isolated by infected animals. Therefore, they represent those multi-resistant microorganisms that it is possible to find and select in a hospital or outpatient environment and that represent one of the main topics associated to the secondary infections, both in the veterinary and public health areas.

The effect of the ozone treatment was assessed on two multidrug resistant gram-positive microorganisms (*Staphylococcus pseudintermedius* and *Enterococcus faecium*), on a multidrug resistant gram-negative strain (*Enterobacter cloacae*) and on a gram-negative strain (*Escherichia coli*).

The results allowed to underline a strong removal of the bacteria with an average reduction of 96,64% and 99,31% for *Staphylococcus pseudintermedius* and *Enterococcus faecium* respectively (Gram +), and an average reduction of 67,27% and 91,68% for *Enterobacter cloacae* and *Escherichia coli* respectively.

The different effectiveness of the treatment on the several bacterial species can be attributed to the difference in the envelope features between Gram + and

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Gram- bacteria, (Gram- less sensitive) and in part attributed to the particular features of resistance of *Enterobacter cloacae*.

The air diffused ozone produced by the Sany Water Plus device allowed to obtain a strong multidrug resistant microorganisms removal in the ideal condition for their growth (surface of cultivation).

Therefore, we believe that the use of a device for an environment sanitization can represent a valid aid to power the effects of the normal sanitization and disinfection of environments, because air diffuse ozone can act on fine dust as well as on surfaces that are difficult to reach during the normal cleaning activities.

Scientific Supervisor

Dr Maria Luisa Menandro

