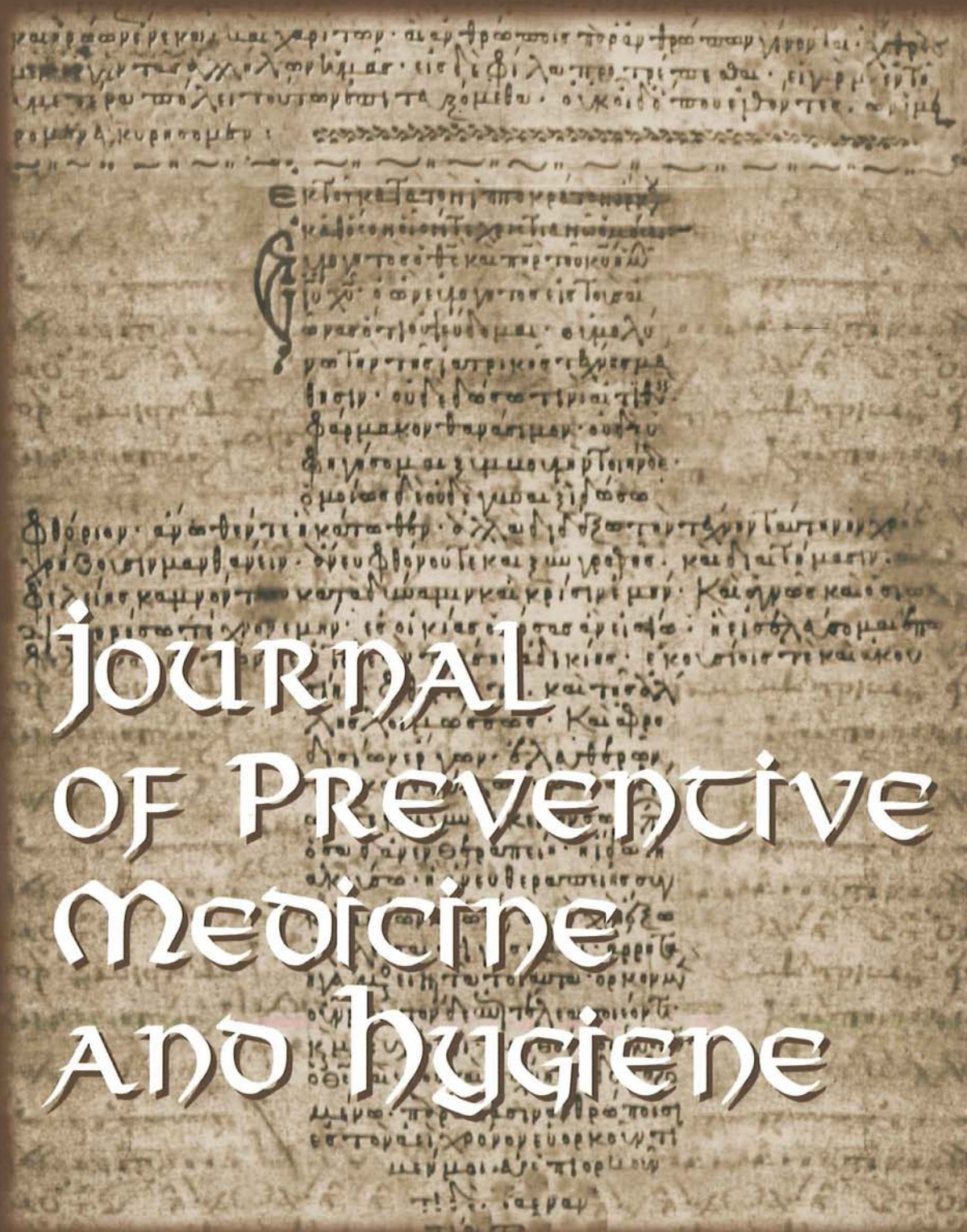


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# Antimicrobial efficacy and longevity of Silver+zeolite incorporating preinsulated ducts installed in real healthcare settings

C. TINTERI, M. POTENZA, R. RIZZETTO

Department of Health Sciences, University of Genoa, Italy

## Key words

HVAC • Insulated Ducts • Zeolite • Silver • Antimicrobial

## Summary

**Objective.** The values of microbial growth in the air exiting from the Heating, Ventilation and Air Conditioning (HVAC) ducts treated with silver/zeolite have been shown to be lower than those in the air coming out the traditional metal ones. This study aims to verify how long this antimicrobial activity lasts.

**Methods.** All the tests were performed according to US ASTM E2180-01 and ISO-JIZ 22196 standards. Samples of aluminum cladding panels of different thickness and incorporating silver-zeolite were tested in order to verify their thickness depending antibacterial activity. The same kind of linings samples were analyzed after a simulated and accelerated ageing process. Ag-zeolite incorporating HVAC duct panels linings were tested after years from their installation, in order to verify the maintenance of their bactericidal power during time.

**Results and conclusions.** For laminates containing different amounts of silver+Zeolite it was shown that also in panels with minimum thickness tested, the lowest germicidal effect (GE) found was still very good (GE of 5,76 ULog10). After their wearing and tearing the antimicrobial activity tended to increase passing from 7.2081 to 8.29922 LogUnits in panels 80microns thick. For still hospital working aluminium foils incorporating Silver/Zeelote on panels installed through 2006 and 2008, the antimicrobial action of zeolite was still firmly present even after two years and three years. The germicidal effect standards were maintained even during time on constant values between 7.477 and 7.086 LogUnits. The persistence of bactericidal efficacy of Ag+zeolite treatment in all the materials used for the construction of HVAC ductworks can be confirmed.

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## Introduction

The bio-contamination of the surfaces is one of the main cause of infections in the community sector (Community Acquired Infections [CAI]) and hospital one (Hospital Acquired Infections [HAI]).

Most of these diseases is transmitted through pathogens in contact with hands or through particulate inhalation and/or aerosol [1, 2].

The growing use of the air conditioning inside the public and private buildings could increase this risk especially when the Heating, Ventilation and Air Conditioning (HVAC) systems are not installed in a proper way or if not regularly serviced [3, 4].

Contaminated air in confined spaces could represent a serious problem for public health also in nosocomial environment especially inside the divisions where heavily immunodepressed patients stay [5].

The standard chemical disinfection of HVAC systems is often difficult because, first, the disinfectants must be correctly applied and they must act for long time; and then the construction materials are often not resistant to corrosion caused by these biocides [6].

Moreover the maintenance plans are often not respected and it is so hard, sometime impossible too, to be able to access all their parts.

Silver and other heavy metals have been used for years as antimicrobial agents in medical, dental, cosmetic and food sectors [7-10].

Previous studies proved that the numbers of microbial colonies present in the outgoing air of a duct microbially treated with Silver/Zeelote were significantly lower than those ones of the traditional metal ducts [11].

The silver/zeolite is an open structure ceramic aluminosilicate that, even with low humidity values, releases silver ions exchanging with Na<sup>+</sup> or Ca<sup>++</sup> ions present in the environment. Thanks to its structure it can be easily incorporated into a wide range of products including the aluminium [12].

This crystalline structure is actually proposed and used as coating on stainless steel surfaces and in the manufacture of air ducts [13].

The new technology with Silver/zeolite incorporation inside the metal or plastic materials intended for panels and HVAC ducts exploits the zeolite property in releasing silver ions in contact with moisture [12].

Particularly the silver ions are able to inhibit the microbial growth through 3 actions: they interfere with the transportation functions, inhibit the respiratory and enzymatic activities and prevent their multiplication by binding with the genetic material [14, 15].

Aim of the research was to test the longevity in time of this bactericidal effectiveness of silver against different microorganisms when silver is incorporated into the zeolite and is released into the environment at ionic state. Its activity against the most known pathogens present in the hospital sector (*Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Legionella pneumophila* and *Aspergillus niger*) has been pursued onto different surfaces previously contaminated, and on samples of panels previously installed in HVAC systems inside some hospital divisions.

The aim was to discover how many log reduction units this microbicide efficacy could reach.

The laboratory tests have been performed on new panels and also on accelerated aged panels according to UNI 10560:1996 and UNI ISO 3248:2001 standards (prolonged thermal and abrasive treatment).

Moreover some specimen have been taken, at scheduled time, from HVAC systems installed from 2006 to 2008 at Gravina (Catania Italy) hospital and Savigliano (Cuneo Italy) hospital in order to verify the duration of the antimicrobial activity over time in health care facilities systems.

## Materials and methods

All the tests have been performed according to US ASTM E2180-01 and ISO-JIZ 22196 standard. The specimens to be tested, having an appropriate concentration of such antimicrobial substance in their composition, were put in contact by their surface with standard amounts of bacterial suspension by means of sterile polyethylene film assuring an optimal distribution.

After a fixed term of incubation inside a thermostat room, the microorganisms present in the solution in contact with the surface were calculated.

The obtained value was compared to that one obtained from the control samples (the control samples are specimen made of the same material but without any bactericidal compound inside their composition; these controls have been contaminated under the same bacterial suspension rates and incubated at the same conditions).

The standard procedure requires the performance of a test together on treated and not treated samples, the use of ATCC strains, the count of cfu/sqcm of surface on not protected samples at time 0 (t0), on not protected samples after 24 hours from the beginning of the test (t24) and on protected samples after 24 hours from the beginning of the test. According to this method every single sample was tested for 6 times against a specific microorganism to avoid overvaluation or undervaluation mistakes due to fortuity.

Moreover the use of a specific neutralizing solution assured that the disinfectant could not be mixed with the recovery culture solutions at times t0 and t24 for the controls and for the examined samples as well.

During a first series of experiments, various silver treated aluminium samples different in thickness were tested, in order to verify if the bactericidal effect was affected by the different amounts of incorporated active ingredient.

Then, other samples with zeolite and silver ions inside were tested; these specimen were subjected to accelerated and simulated ageing process according to UNI 10560:1996 and UNI ISO 3248:2001 standards under a thermal treatment at 150°C for 72h and an abrasive treatment. Samples of the same type of the first ones but not subjected to accelerated ageing were checked too.

Then, treated samples part of panels for HVAC systems in service since several months or years were tested, in order to verify that the bactericidal power remained unchanged over time.

In all cases, untreated samples were contaminated with the same bacterial suspension (positive controls) and untreated samples were not contaminated and tested as negative controls.

For every specimen 3 tiles were checked twice: for each contaminated tile, after the proper incubation time inside in thermostat room (37°C ± 1°C / 24h ± 1h) the count of microorganisms (ufc/ml) still present in every dilution from the recovered liquid was carried out in triple.

All the aluminium panels tested had dimensions of 50 mm x 50 mm and were proved against 5 different strains: *Legionella pneumophila* ATCC 33152, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15422, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC6275). The contamination of every sample was carried out through inoculation of 0,4 ml of bacterial suspension at assayed concentration.

All the samples (except 3 negative controls tested at time zero) were incubated at 37°C ± 1°C for 24 hours and diluted in 10 ml neutralizing broth TSB + LPHI Tween 80 in order to assure that the bactericidal effect was not due to unintentional transport of even infinitesimal disinfectant quantities on the subculture solid medium plates.

From the neutralizing broth, dilutions in ratio 10 were made and aliquots of 0,5ml for every dilution suspension were swarmed in triplicate on Tryptic Soy Agar (TSA). Incubation inside thermostat room (37°C ± 1°C for 24 hours) followed.

The enumeration of the microorganisms, survived on the tested tiles were compared to the microorganisms present on the negative controls at time zero and after 24 hours.

Regarding the moulds and yeasts the incubation temperature used was 25°C ± 1°C for 72 hours and the growth grounds were Sabouraud Dextrose Broth/Sabouraud Dextrose Agar (DSB/ DSA).

## Results

The data obtained related to bacterial load found on different types of samples have been transformed into logarithmic Units. From their comparison, it was obtained the Germicidal Effect (GE) and the corresponding Killing Effect (KE) on the specific tested microorganism.

The study of these parameters (GE and KE) has provided us much more detailed information about the behaviour of the tested substance against various bacterial species, as it better

**Tab. I.** Microbicide efficacy showed on laminates having different thicknesses against stains used for the test.

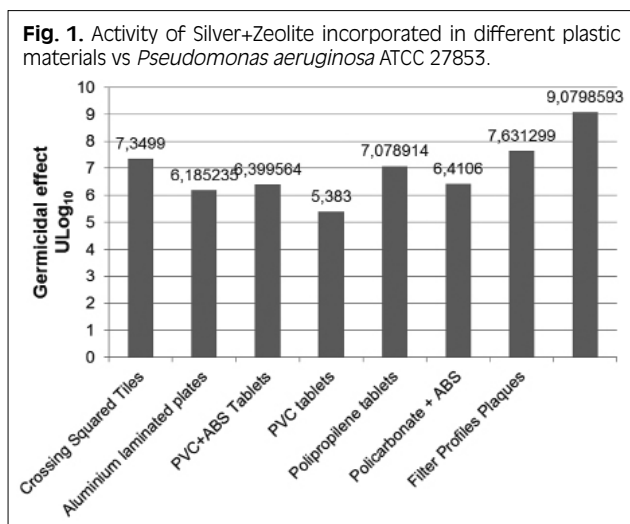
Type of sample	Strain	Control + after 24 h	Sample at 24 h	GE	KE
		CFU/ml	CFU/ml	ULog <sub>10</sub>	%
Laminate 500 µm	Ps. aer 27853	2.29E+07	1.00E-01	6.3419	99.999955
Latten squares 500 µm	Ps. aer 27853	2.29E+07	1.04E+00	5.948717	99.99989
Laminate In-Safe 500 µm	Ps aer 9027	3.60E+08	4.31E+02	5.921448	99.99988
Flat laminate 200 µm	Ps. aer 27853	8.42E+06	3.33E+00	6.623684	99.999978
Laminate 200 µm	Ps aer 9027	5.35E+08	3.45E+03	5.19114	99.9994
Laminate 80 µm	Ps. aer 27853	1.05E+08	1.06E+00	6.993552	99.99999
Laminate 30 µm	Ps. aer 27853	2.82E+07	4.84E+01	5.76498	99.99983
Laminate 30 µm	Ps aer 9027	1.23E+09	1.10E+03	6.048982	99.99991
Laminate 200 µm	L. monocit 19114	1.60E+07	2.00E-01	7.8859	99.999999
Laminate 80 µm	L. monocit 19114	1.60E+07	4.38E+00	6.5637	99.999973
Laminate 80 µm	St. aureus 33862	2.50E+07	1.12E+02	6.287487	99.999948
Laminate 80 µm	C.albicans 2091	4.45E+07	2.43E+02	5.261224	99.999459
Laminate 80 µm	E.coli 25922	1.29E+08	1.73E+02	5.83	99.999855
Laminate 80 µm	L.pneum 3352	1.20E+07	1.67E+00	6.8589	99.999986
Laminate 80 µm	Asp. niger 6275	4.14E+06	1.42E+01	5.4653	99.996

quantified the antimicrobial effect and it did not indicate only the bacteriostatic properties as other methods in use.

Table I shows the values regarding the microbicide efficacy through laminates of different thickness vs different bacterial strains and proportionally protected with different amounts of silver+Zeolite to be used in the inclusion treatment of laminates and plastic materials.

The data show that also in panels with minimum thickness tested against different strains, the lowest GE found was still very good (5.76 ULog<sub>10</sub>), equal to an initial microbial reduction of over 99.999%.

Figure 1 refers to the activity of Silver+Zeolite incorporated in different plastic materials used for fabrication of accessories for HVAC ducts vs *Pseudomonas aeruginosa* ATCC 27853. It is clear that for all the materials there was a minimal GE of 5.383 Logarithmic Units corresponding to a reduction of the original bacterial population of 99.999586%.



**Fig. 1.** Activity of Silver+Zeolite incorporated in different plastic materials vs *Pseudomonas aeruginosa* ATCC 27853.

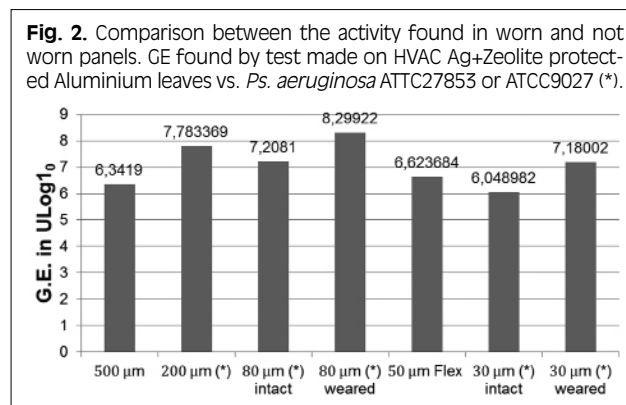
The following Figure 2 shows the laboratory results obtained from panels subjected to simulated ageing compared to panels having the same production date but not subjected to wear.

The data obtained show that in two cases the bactericidal activity has increased passing from 7.2081 to 8.29922 Logarithmic Units in samples with 80 microns thickness and from 6.048982 to 7.18002 Logarithmic Units in panels 30 microns thick.

The third phase of the study was intended to evaluate the efficacy of zeolite incorporated into the panels over the time and consequently, it has been tested the behaviour of panels coming from real installations built between 2006 and 2008 and still working.

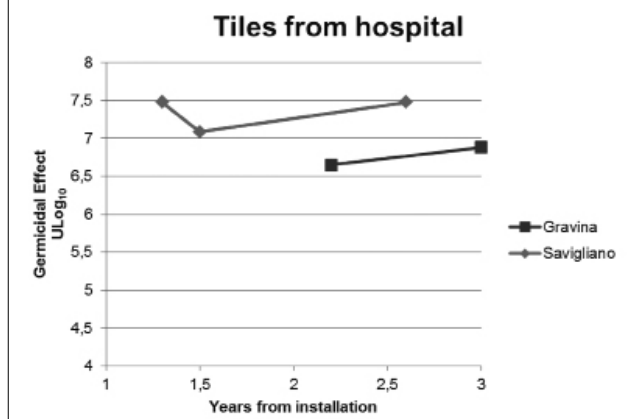
Figure 3 shows the bactericidal activity vs *Pseudomonas aeruginosa* detected after some time on samples of polyurethane panels covered by aluminium foils incorporating Silver+zeolite and installed between 2006 and 2008 in Savigliano Hospital (Cuneo Italy) and Gravina Hospital (Catania Italy).

For both cases it is evident that the antimicrobial action of zeolite is still present even after two years and a half (Savigliano Hospital) and three years (Gravina Hospital).



**Fig. 2.** Comparison between the activity found in worn and not worn panels. GE found by test made on HVAC Ag+Zeolite protected Aluminium leaves vs. *Ps. aeruginosa* ATCC27853 or ATCC9027 (\*).

**Fig. 3.** Bactericidal activity vs *Pseudomonas aeruginosa* detected at different times in two real plants.



The standards of the germicidal effect are maintained even in time on constant values between 7.477 and 7.086 Logarithmic Units in the first case and between 6.653 and 6.884 Logarithmic Units in the second case. These values correspond to a reduction of the bacterial population always over 99.999978%.

## Conclusions

All the tests carried out in the Laboratory of Applied Environmental Microbiology confirmed the excellent bactericidal efficacy of the Ag+zeolite treatment, not only related to pre-insulated panels but also to all the materials used for the construction of HVAC ductworks including profiles, ac-

cessories and consumption materials (glue, sealant, etc). But the main aim of the research was to demonstrate that the antimicrobial efficacy of zeolite surface persists over time. The results obtained both on samples subjected to simulated wear and on samples coming from the operating plants at Savigliano Hospital (Cuneo Italy) and Gravina Hospital (Catania Italy) are extremely positive. The data show that, after three years in one case and after two years and a half in the other one, the antimicrobial performances of treated surfaces were constantly high. Indeed, the percentage of reduction vs *Pseudomonas aeruginosa* was equal to 99.999978% after two years and a half from the installation. This is certainly due to the typical structure of the carrier which releases the active ingredient only in presence of moisture and only when the HVAC system is operating. In practice, when not necessary, for instance, under environmental conditions particularly dry and naturally very unfavourable to bacterial growth, Silver ions are not released; in this way the active principle is consumed very slowly and it is able to carry out its action for many years.

For the future, thanks to the use of mathematical models, we plan to be able to estimate the duration of the antimicrobial efficacy of zeolite treatment in HVAC ductworks, comparing the amount of Silver ions present in the panels of new production with the quantity found in panels for ducts in service for several years.

We also aim to evaluate the germicidal effect against spore-forming bacteria in order to assess on their spores the possible preventive potentiality of silver+zeolite also against spore-forming pathogens bacilla (*Bacillus anthracis*) in the event of their intentional introduction in HVAC ductworks for bio-terrorism purposes.

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n Correspondence: Rolando Rizzetto, via A. Pastore 1, 16135 Genova, Italy. - Tel. +39 010 3538516 - E-mail: rizzetto@unige.it