

**RAPID REDUCTION OF  
*STAPHYLOCOCCUS AUREUS*  
POPULATIONS ON  
STAINLESS STEEL  
SURFACES BY ZEOLITE  
CERAMIC COATINGS  
CONTAINING SILVER AND  
ZINC IONS**



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## Summary

This study demonstrates the anti-*Staphylococcus aureus* properties of stainless steel surfaces coated with zeolite containing 2.5% silver and 14% zinc ions. Stainless steel panels with and without the heavy-metal-containing coatings were inoculated with *S. aureus* and incubated at room temperature. Survival of *S. aureus* was significantly reduced by the silver/zinc coatings within 1 h. Many hospital surfaces could be constructed of stainless steel with silver/zinc zeolite coatings. Such measures may reduce rates of hospital-acquired *S. aureus* infection.

Keywords: *Staphylococcus aureus*; environmental surfaces.

## Introduction

*Staphylococcus aureus* was the most common agent of hospital-acquired infections between 1990 and 1996 in the US.<sup>1</sup> Contaminated inanimate surfaces have been shown to contribute to hospital-acquired infections.<sup>2</sup>

A new antimicrobial material is available that can be bonded to stainless steel surfaces. The material is a zeolite, an aluminum silicate coating containing 2.5% (w/w) silver and 14% zinc ions within the alumino-silicate matrixes (AK Steel Corporation, Middletown, OH, USA). Silver ions inhibit bacterial enzymes, interfere with electron transport, and bind to DNA.<sup>3</sup> Zinc inhibits nutrient uptake and interferes with proton transfer.<sup>4</sup> This study evaluates the use of the silver/zinc coating for the control of *S. aureus* on stainless steel surfaces.

## Materials and methods

All dilutions were performed in 0.85% sterile saline. Numbers of bacteria were determined using the spread plate method. Before each test, *S. aureus* ATCC 25923 (American Type Culture Collection, Rockville, MD, USA) was incubated on tryptic soy agar (Difco, Detroit, MI, USA) at 35°C for 18 h, then 100 mL tryptic soy broth was inoculated and incubated at 35°C on an orbital shaker at 200 rpm for 3 h.

Zeolite powders (AgION Technologies, Wakefield, MA, USA) are composed of grid-like structures forming porous crystals and the ions reside within pores. Powders were supplemented with 2.3% silver, 3.1% silver and 5.4% copper,

or 2.5% silver and 14% zinc ions. Controls were unaltered powders. Suspensions of 0.01% of each powder were made in saline in Erlenmeyer flasks in duplicate. A 1:100 dilution of the 3 h broth culture of *S. aureus* was made into each suspension. Suspensions were incubated at room temperature on an orbital shaker and aliquots placed into Dey Engley (DE) broth (Remel, Lenexa, KS, USA) at designated times. Surviving bacteria were enumerated on mannitol salt agar (MSA, Difco).

Stainless steel panels (10.2 x 10.2 cm) with and without 2.5% silver/14% zinc coatings were disinfected with 70% ethanol. A 1:100 dilution of the *S. aureus* culture was made and 1 mL was placed on each panel. Each inoculum was spread using a sterile cotton-tipped swab and allowed to air dry at room temperature at a relative humidity of 30% (Fisher Hygrometer, Pittsburgh, PA, USA). At 1, 4 and 24 h, surviving *S. aureus* were enumerated by thoroughly swabbing each panel and placing each swab in 1 mL DE broth. The surviving *S. aureus* were enumerated on MSA. Test were performed in duplicate or triplicate.

One-way analysis of variance was performed using Minitab Inc., Release 13 (State College, PA, USA).

## Results

Powder suspension tests were conducted to determine whether the presence of copper or zinc ions would enhance the antibacterial effect of silver alone (Table I). The controls indicated that little removal was due to adsorption by particles. After 4 h incubation, there was a significant die-off of *S. aureus* in supplemented powders compared with the control ( $P = 0.03$ ). However, there were no significant differences between the supplemented powders. Although the effect of zinc or copper alone cannot be evaluated, it is clear that the increased reduction observed with the silver/zinc combination compared with the silver/copper combination is highly significant at 24 h ( $P = 0.009$ ).

Table I Reduction of *Staphylococcus aureus* in zeolite powder amended with varying concentrations of heavy metal ions\*

	Powders			
	Control†	2.3% Ag	3.1% Ag/5.4% Cu	2.5% Ag/14% Zn
Inoculum	4.2x10 <sup>6</sup>	4.2x10 <sup>6</sup>	4.2x10 <sup>6</sup>	4.2x10 <sup>6</sup>
1 h	2.1x10 <sup>6</sup>	4.6x10 <sup>5</sup>	3.9x10 <sup>5</sup>	4.8x10 <sup>5</sup>
4 h	1.5x10 <sup>6</sup>	4.1x10 <sup>5</sup>	3.8x10 <sup>5</sup>	3.8x10 <sup>5</sup>
24 h	1.0x10 <sup>6</sup>	3.8x10 <sup>4</sup>	6.8x10 <sup>2</sup>	1.0x10 <sup>1</sup>

\*Tests were conducted in 0.01% suspensions of powders in 0.85% sterile saline. Results are averages of duplicates expressed as colony-forming units per millilitre of powder suspension.

†Control powder was not supplemented with heavy metals.

Table II Survival of *Staphylococcus aureus*, (colony forming units), on control stainless steel panels and on stainless steel panels with 2.5% silver 14% zinc zeolite coatings incubated at room temperature and 30% relative humidity\*

	Survival of <i>S. aureus</i> after			
	Inoculum on panels	1 h	4 h	24 h
Test 1				
Controls	8.7x10 <sup>4</sup>	4.2x10 <sup>4</sup>	1.3x10 <sup>4</sup>	2.2x10 <sup>3</sup>
Ag/Zn coating	8.7x10 <sup>4</sup>	1.5x10 <sup>2</sup>	1.0x10 <sup>1</sup>	<1.0x10 <sup>1</sup>
Test 2				
Controls	3.9x10 <sup>5</sup>	1.8x10 <sup>5</sup>	9.8x10 <sup>4</sup>	2.8x10 <sup>3</sup>
Ag/Zn coating	3.9x10 <sup>5</sup>	2.4x10 <sup>2</sup>	2.0x10 <sup>1</sup>	<1.0x10 <sup>1</sup>

\* Results are averages of duplicate or triplicate tests.

The survival of *S. aureus* on stainless steel panels, with and without zeolite coatings containing 2.5% silver/14% zinc was evaluated on two occasions (Table II). Some reduction was observed even on control stainless-steel panels. However, die-off of the *S. aureus* was markedly enhanced by the coatings containing silver and zinc. During Test 1, the reduction of *S. aureus* was significantly greater in the presence of the coatings after both 1 h (P = 0.004) and 24 h (P = 0.011) incubation. The differences in survival were even more significant (P<0.001) at both 1 and 24 h during Test 2.

## Discussion

Zeolite powders containing silver or silver with copper or zinc were compared to determine which combinations demonstrated the greatest activity against *S. aureus*. Powder with no heavy metals was used as a control to evaluate the effect of adsorption. The effect of copper or zinc alone was undetermined. Of the ions tested, the

silver/zinc ion combination was the most efficacious. This combination was used in the test coatings for the stainless steel surfaces.

The 2.5% silver/14% zinc coatings have significant anti-*S. aureus* properties. Many types of surfaces in hospitals harbour *S. aureus*.<sup>5</sup> Stainless steel surfaces with heavy metal-supplemented coatings may contribute to the reduction of *S. aureus* infections in the hospital setting. Reduction of bacterial contamination of the patient's environment has been shown to contribute to the control of hospital-acquired *S. aureus* infections.<sup>6</sup> In one outbreak, methicillin-resistant *S. aureus* (MRSA) was isolated from patients, floors, trolleys, light switches, and ventilation ducts. The authors concluded that hand contamination from inanimate objects and airborne contamination contributed to the spread of these resistant strains.<sup>7</sup>

Contaminated duct systems were also implicated as sources of a MRSA outbreak on an orthopaedic ward. An environmental survey showed that ventilation grilles harboured the outbreak strain. It was thought that contaminated air was blown into the unit by the ventilation system. After the air system was cleaned and infection control measures were initiated the outbreak ended.<sup>8</sup>

Investigations of many other hospital-acquired outbreaks suggest that it may be advantageous if environmental surfaces in the hospital setting had antimicrobial properties. Stainless steel surfaces with silver/zinc coatings result in a significant reduction of *S. aureus*. If ventilation ducts, door handles and other surfaces were constructed of stainless steel with silver/zinc coatings, rates of *S. aureus* hospital-acquired infections might decrease.

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