

## Susceptibility of 169 USA300 methicillin-resistant *Staphylococcus aureus* isolates to two copper-based biocides, CuAL42 and CuWB50

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**Objectives:** To test the activity of two copper-based biocides, CuAL42 and CuWB50, and benzalkonium chloride against 169 isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) pulsotype USA300, a virulent, multiply resistant, widespread clone in the USA.

**Methods:** Tests including MIC, MBC and time-kill studies were performed multiple times.

**Results:** The MIC range, MIC<sub>50</sub> and MIC<sub>90</sub> (0.59–18.75, 4.69 and 4.69 ppm, respectively) and the MBC range, MBC<sub>50</sub> and MBC<sub>90</sub> (1.17–18.75, 4.69 and 9.38 ppm, respectively) for CuAL42 were identical with those obtained with CuWB50, except that the MBC range for CuWB50 was wider (0.59–37.5 ppm). In time-kill studies, a 6 log<sub>10</sub> reduction of cfu was achieved within 1 h (150 ppm) and 0.5 h (300 ppm) for CuAL42, and 1.5 h (150 ppm) and 0.75 h (300 ppm) for CuWB50.

**Conclusions:** Both copper-based biocides can effectively kill USA300 MRSA and may facilitate the eradication of the organism from healthcare settings.

**Keywords:** MRSA, disinfectants, time-kill studies, minimum inhibition concentrations

### Introduction

Long regarded as a nosocomial pathogen responsible for severe toxin-mediated disease and invasive pyogenic infections, methicillin-resistant *Staphylococcus aureus* (MRSA) is also recognized as a community-acquired pathogen with increasing antimicrobial resistance and virulence. One community-acquired MRSA (CA-MRSA) clone, identified by the Centers for Disease Control and Prevention as pulsotype USA300, has been implicated in outbreaks within the USA, and accounts for up to 70% of all skin and soft tissue infections presented in emergency departments.<sup>1,2</sup>

Not limited to the community, USA300 can be isolated from newly admitted patients in nursing homes and hospitals. MRSA can colonize or infect other patients, visitors and healthcare workers by direct contact, aerosols and contact with contaminated surfaces.<sup>3</sup> Even if MRSA is eradicated from the patient, it can be found in the environment for long periods of time.<sup>4</sup> Thus, the disinfection of hands and decontamination of surfaces are important steps in infection control.<sup>3,4</sup>

Because increased resistance of MRSA to cleaning agents containing biocides such as quaternary ammonium compounds has been reported,<sup>5</sup> researchers began to investigate other biocides, e.g. copper because of its long-known antimicrobial activity.<sup>6–8</sup> Novel biocidal compounds containing copper have been reported to be effective against nosocomial pathogens.<sup>8,9</sup> However, only a

limited number of MRSA strains have been tested and not the USA300 clone. Therefore, we tested two copper-based biocides, CuAL42 and CuWB50, compared with a representative quaternary ammonium compound, benzalkonium chloride, against 169 isolates of MRSA USA300 from the Center for Biological Defense collection.

### Materials and methods

A total of 169 MRSA isolates previously characterized as USA300 by PFGE were obtained from Washington State and Florida hospitals and outpatient clinics. The 17 MRSA isolates acquired from two hospital laboratories in the Seattle Metropolitan area in Washington State were isolated from cultures of blood, skin lesions and tissue abscesses of patients in 2003 and 2004. The remaining 152 MRSA from the Tampa Bay Metropolitan area in west central Florida were obtained from cultures of abscesses, blood, lesions and nasopharyngeal specimens from patients seen at clinical laboratories, doctors' offices and hospitals from 2004 to 2006. All bacteria were grown on tryptic soy agar supplemented with 5% sheep red blood cells (Remel, Lenexa, KS, USA) overnight at 35 ± 2°C before susceptibility tests were performed.

The copper-based biocides CuAL42 and CuWB50 (described in detail in Gant *et al.*<sup>8</sup>), obtained from Remedy Research Ltd (London, UK), were diluted in RMPI 1640 (Sigma-Aldrich, St Louis, MO, USA), as previously reported.<sup>8</sup> The concentrations of each biocide tested ranged from 0.15 to 300 ppm. Using the microtitre plate format and standard

protocols, both the MIC and MBC were determined for the two biocides.<sup>10</sup> Plates were incubated at  $35 \pm 2^\circ\text{C}$  and read at 24 h. All tests were performed in duplicate. To examine the reproducibility of the test method and stability of the solutions, *S. aureus* ATCC 29213 was included with each group of MRSA tested. Following conventional protocols, the time-kill curve studies were performed using 150 and 300 ppm of both biocides against *S. aureus* ATCC 29213 (CuAL42 and CuWB50 MICs=2.34 ppm) and three USA300 MRSA isolates, each having a different MIC (0.59, 4.69 and 18.75 ppm) to the copper-based biocides.<sup>10</sup> All tests were performed for each isolate and at each concentration a minimum of three times. The mean initial bacterial load used for the time-kill studies was  $3.07 \pm 1.38 \times 10^6$  cfu. The log reduction was determined for each timepoint until no bacterial growth was observed.

For comparison, benzalkonium chloride (Sigma-Aldrich) was prepared in distilled water for MIC and MBC assays (test range of 0.025–2160 ppm). Time-kill curve studies with benzalkonium chloride were performed multiple times at 5, 50, 150 and 300 ppm using the same isolates as with the copper-based biocides.

*S. aureus* 29213 and three USA300 isolates were used for the time-kill curves and tests were performed a minimum of three times for each isolate and each concentration of the tested compound.

## Results

The growth of all 169 CA-MRSA isolates was inhibited by CuAL42, with both the MIC and MBC  $\leq 18.75$  ppm (Table 1). While the growth of most of the isolates was inhibited by CuWB50 at  $\leq 18.75$  ppm, one isolate had an MBC of 37.5 ppm. This is only

one tube dilution (2-fold) different from 18.75 ppm. While the MBC ranges for both copper-based biocides were similar, the MIC<sub>50</sub>, MIC<sub>90</sub>, MBC<sub>50</sub> and MBC<sub>90</sub> values were identical (Table 2). In testing *S. aureus* ATCC 29213 ( $n=11$ ), the median MIC and MBC were 2.34 ppm for both CuAL42 and CuWB50, while the MIC and MBC values for each copper-based biocide ranged from 1.17 to 4.69 ppm (Table 2). The time-kill curve studies demonstrated that 150 ppm of CuAL42 and CuWB50 produced a 6 log<sub>10</sub> reduction in bacterial numbers in 1 and 1.5 h, respectively, for all four isolates tested. Using the 300 ppm concentration of CuAL42 and CuWB50, no bacterial growth was seen at 30 and 45 min, respectively (Table 2).

In contrast, the MIC and MBC values for benzalkonium chloride ranged from 0.1 to 40 ppm, reflecting the growing resistance of *Staphylococcus* spp. to this biocide, as reported previously (Table 2).<sup>4</sup> The times for killing all of the inoculum cells at 150 and 300 ppm were 1 h and <15 min, respectively, while for 50 and 5 ppm this was not achieved within 2 h. When the concentration of benzalkonium chloride was 50 ppm, there was a 2–3 log<sub>10</sub> reduction of bacterial cells at 1 h and 6 log<sub>10</sub> reduction at 2 h. With 5 ppm, there was no appreciable log<sub>10</sub> reduction seen at 2 h.

## Discussion

Because USA300 MRSA now has a predominant presence in both community and hospital settings, it is important to find

**Table 1.** Distribution of MIC and MBC values for 169 isolates of USA300 MRSA

		Biocide concentration (ppm)											
Biocide		<0.15	0.29	0.59	1.17	2.34	4.69	9.38	18.75	37.5	75	150	300
MIC	CuAL42	0	0	1	2	54	103	8	1	0	0	0	0
	CuWB50	0	0	2	19	38	97	12	1	0	0	0	0
MBC	CuAL42	0	0	0	3	35	69	58	4	0	0	0	0
	CuWB50	0	0	2	6	33	69	54	4	1	0	0	0

  

		Biocide concentration (ppm)											
Biocide		<0.10	0.10	0.3	0.625	1.25	2.5	5	10	20	40	80	160
MIC/MBC	BZC	0	1	0	13	48	70	37	0	0	0	0	0

BZC, benzalkonium chloride.

All testing was performed in duplicate.

**Table 2.** MIC and MBC ranges, 50th and 90th percentiles, and total kill times<sup>a</sup> for CuAL42 and CuWB50

Biocide	MIC (ppm)			MBC (ppm)			Time to complete kill	
	range	50%	90%	range	50%	90%	150 ppm	300 ppm
CuAL42	0.59–18.75	4.69	4.69	1.17–18.75	4.69	9.38	1 h	0.5 h
CuWB50	0.59–18.75	4.69	4.69	0.59–37.5	4.69	9.38	1.5 h	0.75 h
BZC	0.10–5	2.5	5	0.10–5	2.5	5	1 h	<0.25 h

BZC, benzalkonium chloride.

<sup>a</sup>All time-kill assays were performed with four strains of *S. aureus* a minimum of three times at each concentration. Each strain had a different MIC of CuAL42 (0.59, 2.34, 4.69 and 18.75 ppm).

disinfectants that can kill the bacterium. Although previous tests with the copper-based biocides CuAL42 and CuWB50 showed effectiveness against hospital pathogens, including limited strains of *S. aureus*, we have now tested the biocides against a large number of USA300 MRSA isolates obtained from patients in both clinical and hospital settings. Our results demonstrate that for each biocide the MIC and MBC values are either identical for a strain or very close to one another (usually only one tube dilution apart; data not shown). Likewise, the MBC ranges for both biocides are very similar. These results are consistent with those obtained in previous studies.<sup>8</sup> In addition, the MICs and MBCs of the two copper-based biocides against the 169 USA300 isolates were clustered around 4.69 ppm. Repeated testing with *S. aureus* ATCC 29213 indicated that the tests were reproducible, because the median test result for both was 2.34 ppm, with only one tube dilution (2-fold) variance in either direction. This conforms with CLSI standards.<sup>10</sup> The MIC and MBC ranges, MIC<sub>50</sub> and MIC<sub>90</sub> obtained for benzalkonium chloride (0.10–5 ppm) were slightly lower than those observed for the two copper biocides, yet were higher than what was expected, because other researchers have reported very low MICs for Gram-positive bacteria with this biocide (0.001–0.6 ppm). This suggests that resistance to benzalkonium chloride may be developing in the MRSA/USA300 population, as has been noted in other *S. aureus* populations.<sup>5</sup>

Both the MIC and MBC values were determined after the bacterial cells were cultured for 24 h. Thus, higher concentrations of the copper-based biocides would be expected to be needed if the killing time is to be shortened, e.g. under circumstances where the copper-based biocides were to be used in a hand gel.<sup>8</sup> When we used 150 and 300 ppm for CuAL42 and CuWB50, 6 log<sub>10</sub> of bacterial cells were killed within the time frame of 30 min to 1.5 h, indicating reasonable working concentrations for both compounds. The copper-based biocides are not toxic to eukaryote cells at these concentrations and are not corrosive to metal surfaces. As a comparison, the quaternary ammonium compound benzalkonium chloride also required 1 h for complete bacterial kill at the 150 ppm concentration. The concentrations of 150 and 300 ppm are much higher than what is usually used with benzalkonium chloride (0.005–40 ppm), perhaps due to its reported toxicity for eukaryotic cells (i.e. skin cells) and corrosive issues mentioned in the Material Safety Data Sheet available from the manufacturer.

Currently, USA300 MRSA is a major concern in both community and hospital settings where the organism can survive for long periods of time on inanimate surfaces. This study suggests that copper-based biocides may be useful in the disinfection of home and hospital surfaces contaminated with this pathogen. In this respect, a cleaning study using CuWB50-impregnated ultramicrofibre cloths and mops was recently shown to reduce bacterial levels on surfaces in hospital wards and to exert a retained kill that kept bacterial levels low between rounds of cleaning.<sup>11</sup>

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