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# Evaluation of the Effectiveness of Common Hospital Hand Disinfectants Against Methicillin-Resistant *Staphylococcus aureus*, Glycopeptide-Intermediate *S. aureus*, and Heterogeneous Glycopeptide-Intermediate *S. aureus*

Mandy Wootton, PhD; Timothy R. Walsh, PhD; Eleri M. Davies, MRCPATH; Robin A. Howe, MRCPATH

**BACKGROUND.** The presence of methicillin-resistant *Staphylococcus aureus* (MRSA) and glycopeptide-intermediate *S. aureus* (GISA) in hospitals poses a significant challenge to hospital infection control teams. The use of disinfectants for both surface and hand cleaning is an essential part of the infection control measures.

**OBJECTIVE.** To evaluate the effectiveness of common hospital hand disinfectants against MRSA, GISA, and heterogeneous GISA (hGISA).

**METHODS.** For methicillin-susceptible *S. aureus* (MSSA), MRSA, GISA, and hGISA, the levels of susceptibility to hand disinfectants and their active ingredients were determined. Suspension tests were performed on commercial handwashing products.

**RESULTS.** Minimum inhibitory concentrations (MICs) of 2-propanol, chlorhexidine, and hexachlorophene were similar for all phenotypes. The MICs of cetrimide and triclosan were higher for the MRSA, GISA, and hGISA strains than for the MSSA strain. The MICs for the chlorhexidine-containing agents Hibisol and Hibiscrub (AstraZeneca) and for the propanol-containing agent Sterillium (Medline) were 1–2-fold lower for the MSSA strains than for the MRSA, GISA, and hGISA strains. Suspension tests showed that the GISA and hGISA strains were less susceptible to the triclosan-containing agent Aquasept (SSL) than were the MRSA and MSSA strains, with resistance increasing with glycopeptide resistance. Products containing Betadine (Purdue) were more effective against the GISA and hGISA strains than against the MRSA and MSSA strains, especially after the strain was exposed to the product for 30 seconds.

**CONCLUSIONS.** Using the EN 1040 standard criteria for the performance of disinfectants, we determined that all agents, except 50% Aquasept for hGISA and 0.33% hexachlorophene for GISA, performed effectively. However, the GISA and hGISA strains were less susceptible to triclosan-containing products, compared with the MRSA strains, but were more susceptible to products containing Betadine.

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*Staphylococcus aureus*—in particular, methicillin-resistant *S. aureus* (MRSA)—is a major cause of hospital-acquired infection. Strains of methicillin-susceptible *S. aureus* (MSSA) are generally susceptible to commonly used hospital disinfectants; however, some MRSA strains have been reported to have reduced susceptibility to chlorhexidine, quaternary ammonium compounds (QACs), cetrimide, and benzalkonium chloride.<sup>1–4</sup> For MRSA, a number of genes (*qacA–D* and *norA*), some carried by plasmids, have been shown to be resistant to chlorhexidine, diamidines, and QACs.<sup>5–8</sup> The presence of *qac* genes is common among MRSA strains and coagulase-negative staphylococci; these genes mediate the efflux-based resistance to QACs.<sup>9</sup> These genes have also been found in a strain of vancomycin-resistant *S. aureus* isolated in the United States.<sup>10</sup> Further reports have shown a ge-

netic linkage between these genes and the antibiotic resistance genes *blaZ*, *aacA–aphD*, *dfrA*, and *ble* on the same plasmids, and concern about the transfer of these genes is understandable.<sup>11–13</sup>

Alternatively, intrinsic resistance to biocides can be conferred by cell-wall thickness and by characteristics associated with biofilm production, such as slow growth rate and altered cell-wall composition.<sup>14</sup> Reduced cellular permeability and components of the cell wall may limit the concentration of active biocides that reach the target site(s).<sup>15</sup> For *Bacillus megaterium*, a thickened and altered peptidoglycan component of the cell walls has demonstrated reduced susceptibility to disinfectants and antiseptics.<sup>16</sup> The cell walls of staphylococci are composed of peptidoglycan and teichoic acid and are not normally an effective barrier against disinfectants or antiseptics.

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TABLE 1. Mean Minimum Inhibitory Concentrations (MICs; mg/L) of Agents Used in Hand Disinfectants, With Levels of Susceptibility of 6 Phenotypes of *Staphylococcus aureus*

Phenotype, MIC	1-propanol	2-propanol	Chlorhexidine	Triclosan	Cetrimide	Hexachlorophene
MSSA ( <i>n</i> = 10)						
MIC range	128	32	0.008–0.015	0.015–2	2–4	0.25–0.5
MIC <sub>50</sub>	128	32	0.008	0.015	2	0.5
MIC <sub>90</sub>	128	32	0.015	0.5	2	0.5
EMRSA-15 ( <i>n</i> = 10)						
MIC range	128	32	0.015	0.015–0.03	8–16	0.25–0.5
MIC <sub>50</sub>	128	32	0.015	0.03	8	0.5
MIC <sub>90</sub>	128	32	0.015	0.03	8	0.5
EMRSA-16 ( <i>n</i> = 10)						
MIC range	128	32	0.015	0.015	2–32	0.25–1
MIC <sub>50</sub>	128	32	0.015	0.015	16	0.5
MIC <sub>90</sub>	128	32	0.015	0.015	16	0.5
MRSA ( <i>n</i> = 16)						
MIC range	64–128	32–64	0.008–0.06	0.015–4	2–16	0.25–1
MIC <sub>50</sub>	128	32	0.015	0.03	8	0.5
MIC <sub>90</sub>	128	64	0.03	2	8	0.5
hGISA ( <i>n</i> = 45)						
MIC range	16–128	32–64	0.008–0.03	0.015–2	4–32	0.12–0.5
MIC <sub>50</sub>	32	32	0.03	1	16	0.25
MIC <sub>90</sub>	32	32	0.03	2	16	0.5
GISA ( <i>n</i> = 8)						
MIC range	16–32	32–64	0.008–0.03	0.015–4	2–16	0.25–0.5
MIC <sub>50</sub>	32	32	0.015	0.015	8	0.25
MIC <sub>90</sub>	32	64	0.015	2	16	0.5

NOTE. The MIC<sub>50</sub> and MIC<sub>90</sub> values are the MICs required to inhibit 50% and 90% of the organisms, respectively. EMRSA-15, epidemic strain of type 15 methicillin-resistant *S. aureus* (MRSA); EMRSA-16, epidemic strain of type 16 MRSA; GISA, glycopeptide-intermediate *S. aureus*; hGISA, heterogeneous GISA; MSSA, methicillin-susceptible *S. aureus*.

tics.<sup>17</sup> For clinical strains of *S. aureus* with intermediate resistance to glycopeptide (hereafter referred to as glycopeptide-intermediate *S. aureus* [GISA]) or with heterogeneous GISA (hereafter referred to as hGISA), the cell wall is grossly thickened with altered cross-linking, and it is possible that these alterations in the cell wall may affect susceptibility to disinfectants.

The emergence of GISA strains and the more abundant hGISA strains is likely to pose a major challenge to hospital infection control teams; with enhanced infection control measures already in operation in many hospitals, the need for accurate data on the efficacy of disinfectant agents against these strains is paramount. A cornerstone of these infection control measures will be effective hand disinfection to prevent the spread of infection from patient to patient, either by healthcare workers or visiting persons. In our study, we evaluated the effectiveness of the cleaning agents used in hand disinfectants and of commercially available hand disinfection products against a range of *S. aureus* strains with differing levels of susceptibility to methicillin and glycopeptide. Minimum inhibitory concentrations (MICs) of cleaning agents were determined, and suspension tests were employed to mimic the use of hand disinfectants.

## METHODS

### MICs of Cleaning Agents

The MICs were determined for the following cleaning agents, by the use of the Clinical Laboratory Standards Institute recommended methods and Mueller-Hinton agar plates with log 2 dilutions (from 0.008 to 128 mg/L): povidone-iodine (a halogen-releasing agent), triclosan (a bisphenol compound), cetrimide (a QAC), hexachlorophene (a bisphenol compound), 1-propanol (an alcohol), 2-propanol (an alcohol), and chlorhexidine (a biguanidine derivative). Also tested were the following commercial hand disinfectant products, which were also placed in Mueller-Hinton agar with log 2 dilutions: Hibisol (2.5% chlorhexidine gluconate) and Hibiscrub (20% chlorhexidine gluconate) from AstraZeneca; Sterillium (45% 2-propanol, 30% 1-propanol, and 0.2% mectronium etil-sulphate) from Medline; Aquasept (2% triclosan, diluted to give a range of 0.0015%–0.08%) and Manusept (0.5% triclosan, diluted to give a range of 0.0006%–0.6%) from SSL; and Betadine skin cleanser (4% povidone-iodine), Betadine aqueous solution (10% povidone-iodine), and Betadine alcoholic solution (10% povidone-iodine in alcohol) from Purdue.<sup>18</sup>

TABLE 2. Mean Minimum Inhibitory Concentrations (MICs; mg/L) of Commercially Available Hand Disinfectants, With Levels of Susceptibility of 4 Phenotypes of *Staphylococcus aureus*

Phenotype, MIC	Hibisol	Hibiscrub	Sterillium	Aquasept	Manusept	Betadine		
						Skin cleanser	Aqueous solution	Alcoholic solution
MSSA ( <i>n</i> = 10)								
MIC range	0.03	0.0015	0.015–0.06	0.0012 to >0.6	0.0012 to >0.08	0.12–0.5	2.5	2.5
MIC <sub>50</sub>	0.03	0.0015	0.015	0.0012	0.0025	0.5	2.5	2.5
MIC <sub>90</sub>	0.03	0.0015	0.03	>0.6	>0.08	0.5	2.5	2.5
MRSA ( <i>n</i> = 10)								
MIC range	0.03–0.06	0.003	0.03–0.25	0.0012	0.0012–0.005	0.03–0.5	2.5	2.5
MIC <sub>50</sub>	0.06	0.003	0.12	0.0012	0.0025	0.06	2.5	2.5
MIC <sub>90</sub>	0.06	0.003	0.12	0.0012	0.005	0.5	2.5	2.5
hGISA ( <i>n</i> = 11)								
MIC range	0.03–0.12	0.0015–0.006	0.03–0.25	0.0012–0.3	0.0025 to >0.08	0.03–0.5	2.5–5	2.5
MIC <sub>50</sub>	0.06	0.003	0.12	0.0012	0.0025	0.5	2.5	2.5
MIC <sub>90</sub>	0.06	0.003	0.25	0.3	>0.08	0.5	2.5	2.5
GISA ( <i>n</i> = 10)								
MIC range	0.03–0.06	0.0015–0.006	0.03–0.25	0.0012–0.3	0.0025 to >0.08	0.03–0.5	2.5	2.5
MIC <sub>50</sub>	0.03	0.0015	0.12	0.0012	0.0025	0.5	2.5	2.5
MIC <sub>90</sub>	0.06	0.003	0.25	0.3	0.08	0.5	2.5	2.5

NOTE. The MIC<sub>50</sub> and MIC<sub>90</sub> values are the MICs required to inhibit 50% and 90% of the organisms, respectively; the MICs of these commercially available hand disinfectants were placed on Mueller-Hinton agar plates with log 2 dilutions. GISA, glycopeptide-intermediate *S. aureus*; hGISA, heterogeneous GISA; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

The strains tested were 10 strains of MSSA, 10 epidemic strains of type 15 MRSA, 10 epidemic strains of type 16 MRSA, 16 strains of MRSA, 45 strains of hGISA, and 8 strains of GISA (plus the control *S. aureus* strain ATCC 6538). Two sets of related hGISA and GISA strains were included in the strain sets used: LIM1 (hGISA) and LIM2 and LIM3 (GISA); and PC1 (hGISA) and PC2 (GISA).

### Suspension Tests

Suspension tests were performed with agent concentrations (ie, at 100% concentration and/or at 50% concentration but diluted with water) and recorded times (ie, for handwashing duration) in a way that mimicked the actual use of hand disinfectants. Suspension tests were performed, according to the Council of Europe and the European standard EN 1040, on the commercial agents Hibiscrub (used at 10%), Hibisol (used at 100% and 50%), Aquasept (used at 50%), Manusept (used at 50%), Sterillium (used at 100% and 50%), Betadine antiseptic solution (used at 100% and 50%), Betadine alcoholic solution (used at 100% and 50%), Betadine skin cleanser (used at 100% and 50%), and 0.33% hexachlorophene (used at a 10% concentration of the commercially available 3% soap).<sup>19,20</sup>

The disinfectants were inactivated during suspension tests by the use of one of the following neutralizing fluids:

1. 3 g lecithin (Sigma), 30 mL Tween 80 (Sigma), 5 g Na thiosulphate (BDH), 10 mL phosphate buffer, and 1 g l-histidine (Sigma) in 1 L of water (for Betadine alcoholic solution and Betadine skin cleanser).

2. 3 g lecithin, 30 mL Tween 80, 5 g Na thiosulphate, 10 mL phosphate buffer, and 30 g saponine (Sigma) in 1 L of water (for Aquasept, Manusept, and 0.33% hexachlorophene).

3. 30 mL Tween 80, 4 g sodium dodecyl sulfate, 3 g lecithin, and 10 mL phosphate buffer in 1 L of water (for Hibiscrub, Hibisol, and Sterillium).

Each disinfectant solution (ie, 8 mL) was mixed with 1 mL of water of standard hardness (prepared by adding 3 mL of a solution of 31.74 g magnesium chloride and 73.99 g calcium chloride in 1 L of water to 4 mL of a solution of 56.03 g sodium bicarbonate [BDH] in 1 L of water) and 1 mL of test bacterial suspension (approximately  $1.5 \times 10^8$  organisms) (prepared by adding 0.5 McFarland suspension from culture plates to diluents of 1 g peptone [Sigma] or 8.5 g sodium chloride [BDH] in 1 L water) and incubated for 30, 60, 120, and 600 seconds at room temperature. Simultaneously, 1 mL of bacterial suspension was added to 9 mL of water of standard hardness and spread onto a tryptone soya agar plate for initial inoculum calculations. These samples were removed and added to 1 mL of water of standard hardness and 8 mL of neutralizing fluid to inactivate disinfectant activity. After 5 minutes at room temperature, 500  $\mu$ L of the bacterial suspension samples were spread onto duplicate tryptone soya agar plates, and these plates were incubated in air at 37°C for 24 hours. The surviving bacteria were counted, and a cleaning agent was deemed to be effective against the organism tested if the reduction in the number of viable surviving colonies (ie, the log reduction factor) was at least  $10^5$  colony-forming units/mL within 5 minutes, according to the criteria of European and British standards.

TABLE 3. Effectiveness of Commercial Handwashing Products Against 4 Phenotypes of *Staphylococcus aureus*, Based on Log Reduction Factors (LRFs)

Agent, phenotype	LRF, by exposure time			
	30 sec	60 sec	120 sec	600 sec
Betadine aqueous 100%				
MSSA	2.47	3.79	5.84	5.84
MRSA	2.1	3.46	5.82	5.82
hGISA	2.37	3.29	5.54	5.85
GISA	2.05	3.15	5.81	5.81
Betadine aqueous 50%				
MSSA	1.28	3.04	5.99	6.06
MRSA	0.61	2.23	5.6	5.75
hGISA	2.15	3.83	5.7	5.7
GISA	1.95	4.26	5.64	5.69
Betadine alcohol 100%				
MSSA	5.45	5.85	5.87	5.87
MRSA	5.42	5.7	5.83	5.83
hGISA	5.79	5.84	5.84	5.84
GISA	5.08	5.75	5.75	5.75
Betadine alcohol 50%				
MSSA	1.74	1.92	5.89	5.89
MRSA	1.49	1.94	5.81	5.81
hGISA	2.71	3.56	5.67	5.91
GISA	1.47	1.82	4.64	5.78
Betadine skin cleanser 100%				
MSSA	1.92	3.47	5.81	5.84
MRSA	2.74	4.64	5.75	5.75
hGISA	3.38	4.95	5.46	5.46
GISA	3.27	4.62	5.51	5.51
Betadine skin cleanser 50%				
MSSA	1.55	2.21	5.9	5.9
MRSA	2.01	3.18	5.97	5.97
hGISA	1.57	2.67	5.66	5.86
GISA	1.15	2.31	5.75	5.91
Hibiscrub 10%				
MSSA	5.39	5.88	5.91	5.91
MRSA	4.95	5.49	5.87	5.87
hGISA	5.05	5.71	5.75	5.75
GISA	4.91	5.98	5.99	5.99
Hibisol 100%				
MSSA	5.87	5.87	5.87	5.87
MRSA	5.85	5.85	5.85	5.85
hGISA	5.93	5.93	5.93	5.93
GISA	5.87	5.87	5.87	5.87
Hibisol 50%				
MSSA	5.99	6.02	6.02	6.02
MRSA	5.87	5.89	5.89	5.89
hGISA	5.91	5.91	5.91	5.91
GISA	5.78	5.84	5.84	5.84
Aquasept 50%				
MSSA	3.95	4.14	5.34	5.88
MRSA	3.95	4.39	5.43	5.92
hGISA	2.68	3.19	3.61	4.47
GISA	3.7	3.96	4.95	5.87
Manusept 50%				
MSSA	5.38	5.66	5.91	5.91

(continued)

TABLE 3. (Continued)

Agent, phenotype	LRF, by exposure time			
	30 sec	60 sec	120 sec	600 sec
MRSA	5.27	5.86	5.86	5.86
hGISA	4.57	5.05	5.26	5.47
GISA	5.85	5.85	5.85	5.85
Sterillium 100%				
MSSA	5.94	5.94	5.94	5.94
MRSA	5.72	5.72	5.72	5.72
hGISA	5.57	5.66	5.66	5.66
GISA	5.51	5.51	5.51	5.51
Sterillium 50%				
MSSA	4.33	5.49	5.86	5.95
MRSA	4.9	5.79	5.97	5.97
hGISA	4.95	5.96	5.97	5.97
GISA	3.25	4.98	5.99	5.99
Hexachlorophene 0.33%				
MSSA	2.28	2.46	4.46	5.92
MRSA	1.71	2.19	3.82	5.33
hGISA	2.13	2.69	3.75	5.53
GISA	2.23	2.56	3.71	4.99

NOTE. GISA, glycopeptide-intermediate *S. aureus*; hGISA, heterogeneous GISA; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

Colony counts at 30 seconds were used to mimic the actual time it takes to use a hand disinfectant. The recommendation by the Healthcare Infection Control Practices Advisory Committee for duration of hand washing is currently 15 seconds; however, a time of 30 seconds was selected as the minimum time tested efficiently in vitro.

## RESULTS

### MICs of Cleaning Agents

The MICs of the cleaning agents used in hand disinfectants are shown in Table 1. The MICs of 2-propanol, chlorhexidine, and hexachlorophene were similar for all phenotypes. In contrast, the MIC<sub>50</sub> and MIC<sub>90</sub> values of 1-propanol for GISA and hGISA were 2-fold lower than those for MRSA and MSSA, which suggests that GISA and hGISA are more susceptible to 1-propanol. In contrast, the susceptibility to the hand disinfectant Sterillium containing 45% 2-propanol, 30% 1-propanol, and 0.2% mecetronium etilsulphate was similar for MRSA, GISA, and hGISA but lower for MSSA.

For MRSA, GISA, and hGISA, the cetrinide and triclosan MIC<sub>50</sub> values were 2–4-fold higher than those for MSSA, and the cetrinide and triclosan MIC<sub>90</sub> values were 2–8-fold higher than those for MSSA. The levels of susceptibility to commercially available hand disinfectants were similar for all strains, regardless of the vancomycin resistance phenotype (Table 2). However, the MICs of the chlorhexidine-containing agents Hibisol and Hibiscrub and the propanol-containing Sterillium were 1–2-fold lower for MSSA than for MRSA, GISA, and hGISA.

TABLE 4. Mean Log Reduction Factors Used to Determine the Effectiveness of 9 Commercial Handwashing Products (After 30 Seconds of Exposure) Against 4 Phenotypes of *Staphylococcus aureus*

Phenotypes	Betadine aqueous	Betadine alcohol	Betadine skin	Hibiscrub	Hibisol	Aquasept	Manusept	Sterillium	Hexachlorophene
MSSA and MRSA	1.62	3.53	2.06	5.17	5.93	3.95	5.33	4.62	1.99
hGISA and GISA	2.13	3.77	2.35	4.98	5.85	3.19	5.21	4.1	2.18

NOTE. GISA, glycopeptide-intermediate *S. aureus*; hGISA, heterogeneous GISA; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

### Suspension Tests

According to the EN 1040 standard criteria for the performance of disinfectants, all cleaning agents, except 50% Aquasept for hGISA and 0.33% hexachlorophene for GISA, were effective, with log reduction factors of 5 or more at 600 seconds (ie, at 10 minutes). With the use of Aquasept at 50% strength, the hGISA strains had a mean log reduction factor of 4.47 that was equivalent to a kill rate of 74.6% in 5 minutes. This suggests that hGISA strains are slightly more resistant to this agent than are the MRSA, MSSA, and GISA strains, with log reduction factors of 100%, 100%, and 100%, respectively. This correlates well with the higher MICs of triclosan found for hGISA (Tables 1 and 3). For GISA strains, the log reduction factor achieved with 0.33% hexachlorophene was 4.99, which suggests a slight lowering of susceptibility to this agent.

### DISCUSSION

Concern has been steadily growing with regard to the potential coresistance to antibiotics and disinfectants of clinically

important bacteria. The dominance of MRSA and the emerging threat posed by GISA and hGISA in the clinical setting heighten the importance of hand disinfection as an infection control measure. The altered cell-wall physiology seen in strains of GISA and hGISA, and their increased resistance to glycopeptides, may be related to resistance to disinfectants; however, it is unlikely to be the sole factor.

In our study, the MICs of the hand disinfection agents 2-propanol, chlorhexidine, and hexachlorophene were similar for all phenotypes, which indicates that there are no significant differences between the levels of susceptibility for the GISA, hGISA, MRSA, and MSSA strains. In contrast, the levels of susceptibility to cetrimide were lower for the MRSA, GISA, and hGISA strains than they were for the MSSA strains, which confirms previous reports of reduced susceptibility to QACs observed for MRSA, with MICs that were 1.5–3-fold higher, compared with those for MSSA.<sup>21</sup> For MRSA strains, there is the possible presence of QAC resistance *qac* genes, and, for QAC-resistant biofilm-associated cells, slow growth rates were shown to be the predominant resistance factor.<sup>22</sup>

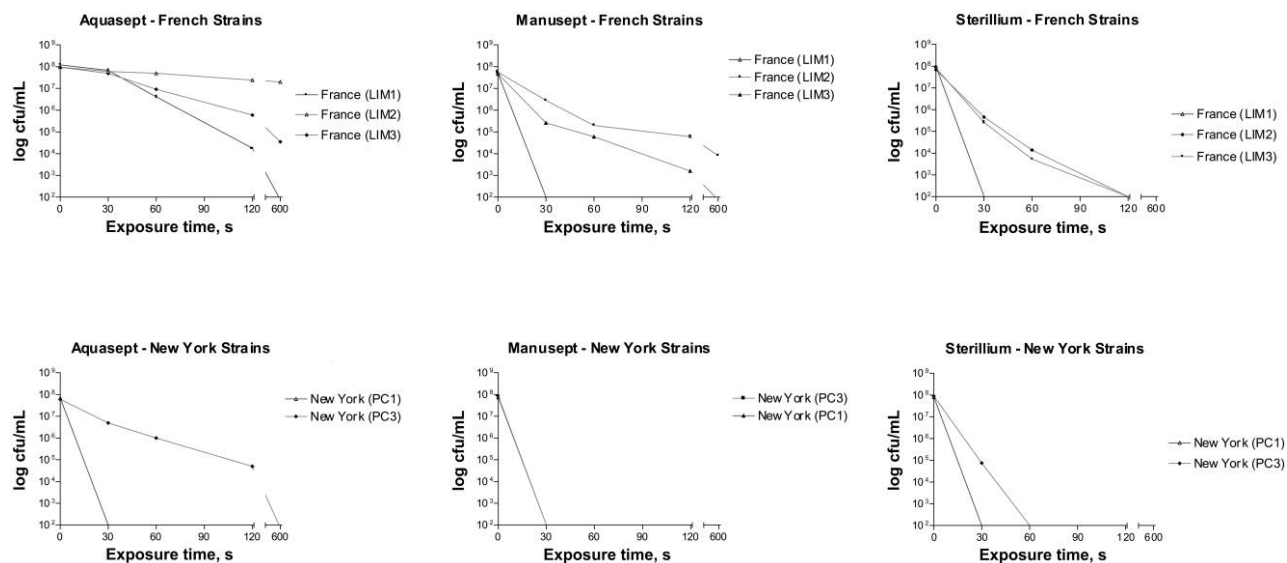


FIGURE. Efficacy of Aquasept, Manusept, and Sterillium against related strains of *Staphylococcus aureus* with intermediate resistance to glycopeptide and with heterogeneous intermediate resistance to glycopeptide. For the bacterial suspension tests, the surviving bacteria were counted, and a cleaning agent was deemed to be effective against the organism tested if the reduction in the number of viable surviving colonies (ie, the log reduction factor) was  $10^5$  colony-forming units (cfu)/mL or more within 5 minutes, according to the criteria of European and British standards.

All GISA and hGISA strains exhibited slower growth rates, compared with the MRSA strains, and have been associated with contamination of biomedical devices, which suggests an ability to produce biofilms and therefore a tendency toward resistance to QACs.<sup>23,24</sup> QACs are commonly used in skin disinfectant products, and, because GISA and hGISA strains show the same propensity for resistance as do MRSA strains, care should be taken whenever these compounds are used for effective skin disinfection.

Triclosan susceptibility was also reduced for MRSA, GISA, and hGISA, compared with MSSA, with MIC<sub>90</sub> values of 2 mg/L and with some isolates having triclosan MICs of 2 and 4 mg/L. These data support previous reports of triclosan-resistant strains of *S. aureus*, with triclosan MICs of 2–4 mg/L and of 32 mg/L.<sup>25,26</sup> Triclosan-containing preparations, such as Aquasept, are regularly used in baths at concentrations of 4–5 mg/L to eradicate MRSA from carrier patients. However, in the case of resistant isolates having triclosan MICs of 32 mg/L, and also those with low-level resistance (ie, 4 mg/L), the triclosan concentration in the bath water would not be sufficient to eradicate the microbe.<sup>26</sup> In our study, no epidemic strains of type 16 MRSA exhibited elevated triclosan MICs, as found in previous reports and in contrast to a report in 2002 that showed triclosan resistance occurring in epidemic strains of type 16 MRSA.<sup>27,28</sup> The reduced levels of susceptibility to the chlorhexidine-containing agents Hibisol and Hibiscrub found for MRSA, GISA, and hGISA, compared with those found for MSSA, confirm the association between methicillin resistance and chlorhexidine resistance.<sup>1,21</sup>

Biocide concentrations used for disinfection and hand sterilization are often varied, with initial applications usually being above MICs. However, these concentrations are lowered by the process of dilution and/or by the duration of time spent hand washing. For example, in the process of hand washing, the concentration of the disinfectant is diluted by the amount of water used. However, the lack of correlation between biocide MICs and their lethal effects has been reported previously.<sup>25,29,30</sup> By the use of the mean log reduction factor and percentage kill at the time tested for hand washing (30 seconds), the effectiveness of these agents was assessed for MSSA and MRSA and for hGISA and GISA (Table 4). The average duration of hand washing in a healthcare setting varied from 4.7 to 24 seconds, with the recommendation (by the Healthcare Infection Control Practices Advisory Committee) in the United States being 15 seconds. The data obtained allowed us to determine whether the intermediate vancomycin resistance phenotype, with its altered physiological features, had any effect on bacterial viability after hand washing (of less than 5-minute exposure).

By using this type of analysis, we showed that all Betadine products containing 4%–10% povidone-iodine had a higher log reduction factor for hGISA and GISA strains than for MSSA and MRSA strains, which suggests that the hGISA and GISA strains were more susceptible to these agents (Table 4). The chlorhexidine-containing agents, Hibisol and Hibiscrub,

were very effective against all strains at 30 seconds, irrespective of vancomycin susceptibility phenotype. Hibisol was slightly more effective, probably due to the additive effect of the isopropanol base. After 30 seconds of exposure to Aquasept (containing triclosan), and according to the EN 1040 standard criteria, the hGISA and GISA strains showed a lower log reduction factor than did the MSSA and MRSA strains (ie, 3.19 vs 3.95); therefore, Aquasept is less effective against the hGISA and GISA strains than against the MSSA and MRSA strains at 30 seconds (Figure). However, no difference was found between strains exposed to Manusept. This could be due to the additive effect of the isopropanol base, as seen for Hibisol, compared with Hibiscrub. When related hGISA and GISA strains were present, the efficacy of Aquasept, Manusept, and Sterillium were compared (Figure), and the data clearly show a reduction in susceptibility to these agents with increasing glycopeptide resistance. In the case of hexachlorophene, the hGISA and GISA strains were more susceptible than the MSSA and MRSA strains at 30 seconds, despite the agent being less effective against the former strain types after 600 seconds of exposure.

In summary, infection control to prevent the spread of MRSA infection in hospitals has focused on effective hand disinfection. Although most hand disinfectants remain active irrespective of the glycopeptide resistance phenotype, from the result of our study, it seems that GISA and hGISA strains are less susceptible to triclosan. On the other hand, Betadine agents were more effective against GISA and hGISA strains, especially after 30 seconds of exposure. Whether it is likely that the altered physiology of hGISA and GISA strains, compared with the vancomycin-susceptible MRSA and MSSA strains, accounts for the differences in the effectiveness of disinfection agents seen in our study is difficult to say. It is therefore important to investigate the exact genetic alterations taking place in these strains, so as to provide information for the purpose of combating disease and the spread of disease caused by these organisms that are resistant to certain cleaning agents.

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#### REFERENCES

1. Irizarry L, Merlin T, Rupp J, et al. Reduced susceptibility of methicillin-resistant *Staphylococcus aureus* to cetylpyridinium chloride and chlorhexidine. *Chemotherapy* 1996; 42:248-252.
2. Kampf G, Jarosch R, Rüdten H. Limited effectiveness of chlorhexidine

- based hand disinfectants against methicillin-resistant *Staphylococcus aureus* (MRSA). *J Hosp Infect* 1998; 38:297-303.
3. Al-Masaudi SB, Day MJ, Russell AD. Sensitivity of methicillin-resistant *Staphylococcus aureus* to some antibiotics, antiseptics and disinfectants. *J Appl Bacteriol* 1988; 65:329-337.
  4. Akimitsu N, Hamamoto H, Inoue R, et al. Increase in resistance of methicillin-resistant *Staphylococcus aureus* to  $\beta$ -lactams caused by mutations conferring resistance to benzalkonium chloride, a disinfectant widely used in hospitals. *Antimicrob Agents Chemother* 1999; 43:3042-3043.
  5. Smith K, Gemmell CG, Hunter IS. The association between biocide tolerance and the presence or absence of *qac* genes among hospital-acquired and community-acquired MRSA isolates. *J Antimicrob Chemother* 2008; 61:78-84.
  6. Littlejohn TG, DiBerardino D, Messerotti LJ, Spiers SJ, Skurray RA. Structure and evolution of a family of genes encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *Gene* 1991; 101:59-66.
  7. Grinius L, Dreguniene G, Goldberg EB, et al. A staphylococcal multidrug resistance gene product is a member of a new protein family. *Plasmid* 1992; 27:119-129.
  8. Noguchi N, Hase M, Kitta M, Sasatsu M, Deguchi K, Kono M. Antiseptic susceptibility and distribution of antiseptic-resistance genes in methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol Lett* 1999; 172:247-253.
  9. Sidhu MS, Heir E, Leegaard T, et al. Frequency of disinfectant resistance genes and genetic linkage with  $\beta$ -lactamase transposon *Tn552* among clinical staphylococci. *Antimicrob Agents Chemother* 2002; 46:2797-2803.
  10. Weigel LM, Clewell DB, Gill SR, et al. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science* 2003; 302:1569-1571.
  11. Berg T, Firth N, Apisiridej S, Hettiaratchi A, Leelaporn A, Skurray RA. Complete nucleotide sequence of pSK41: evolution of staphylococcal conjugative multiresistant plasmids. *J Bacteriol* 1998; 180:4350-4359.
  12. Lyon BR, Skurray R. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol Rev* 1987; 51:88-134.
  13. Paulsen IT, Gillespie MT, Littlejohn TG, et al. Characterisation of *sin*, a potential recombinase encoding gene of *Staphylococcus aureus*. *Gene* 1994; 141:109-114.
  14. Brown MR, Gilbert P. Sensitivity of biofilms to antimicrobial agents. *J Appl Bacteriol* 1993; 74 Suppl:87S-97S.
  15. Russell AD. Mechanisms of bacterial resistance to biocides. *Int Biodeterior Biodegrad* 1995; 36:247-265.
  16. Gilbert P, Brown MR. Some perspectives on preservation and disinfection in the present day. *Int Biodeterior Biodegrad* 1995; 36:219-226.
  17. Russell AD, Russell NJ. Biocides: activity, action and resistance. *Symp Soc Gen Microb* 1995; 53:327-365.
  18. CLSI. Performance standards for antimicrobial susceptibility testing. CLSI document. Wayne, PA: CLSI, 2003:M100-S13.
  19. Council of Europe. Test methods for the antimicrobial activity of disinfectants in food hygiene. Strasbourg, France: Council of Europe, 1987.
  20. British Standard EN 1040:1997. Chemical disinfectants and antiseptics. Basic bactericidal activity—test method and requirements (phase 1). 1997.
  21. Suller MT, Russell AD. Antibiotic and biocide resistance in methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. *J Hosp Infect* 1999; 43:281-291.
  22. Evans DJ, Allison DG, Brown M, et al. Growth rate and the resistance of gram-negative biofilms to cetrимide. *J Antimicrob Chemother* 1990; 26:473-478.
  23. Pfeltz RF, Singh VK, Schmidt JL, et al. Characterisation of passage-selected vancomycin-resistant *Staphylococcus aureus* strains of diverse parental backgrounds. *Antimicrob Agents Chemother* 2000; 44:294-303.
  24. Sakoulas G, Eliopoulos GM, Moellering RC Jr, et al. Accessory gene regulator (*agr*) locus in geographically diverse *Staphylococcus aureus* isolates with reduced susceptibility to vancomycin. *Antimicrob Agents Chemother* 2002; 46:1492-1502.
  25. Cookson BD. Antiseptic resistance in methicillin-resistant *Staphylococcus aureus*: an emerging problem. In: *Proceedings of the Seventh International Symposium*. Stockholm, Sweden; 1994:227-234.
  26. Bamber AI, Neal TJ. An assessment of triclosan susceptibility in methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. *J Hosp Infect* 1999; 41:107-109.
  27. Al-Doori Z, Morrison D, Edwards G, Gemmell C. Susceptibility of MRSA to triclosan. *J Antimicrob Chemother* 2003; 51:185-186.
  28. Brenwald NP, Fraise AP. Triclosan resistance in methicillin-resistant *Staphylococcus aureus* (MRSA). *J Hosp Infect* 2003; 55:141-144.
  29. Cookson BD, Bolton MC, Platt JH. Chlorhexidine resistance in *Staphylococcus aureus* or just an elevated MIC? An in vitro and in vivo assessment. *Antimicrob Agents Chemother* 1991; 35:1997-2002.
  30. Farrelly HD, Stapleton P, Garvey RP, Price MR, Cookson BD. Transferable triclosan resistance in methicillin-resistant *Staphylococcus aureus*. In: "Clinical Microbiology New Perspectives," Joint Meeting of the Association of Medical Microbiology, British Society for Antimicrobial Chemotherapy, Hospital Infection Society and Irish Society of Clinical Microbiology. Dublin, Ireland; 1992. Abstract 41.