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SUMMARY

The activity of eleven disinfectants against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Saccharo-myces cerevisiae* was determined using a method based on the A.O.A.C. germicidal and detergent sanitizer assay. Based on the activity against the test organisms after 30- and 60-s exposures to each disinfectant, the disinfectant containing chlorine dioxide had the highest biocidal activity in this assay, on a mg/l basis. In addition, a disinfectant containing sodium hypochlorite and a disinfectant containing sodium chlorite performed well, at concentrations below label specifications. The results illustrate the importance of testing disinfectants in the context of their intended use.

INTRODUCTION

Disinfection of hard surfaces is an important aspect of the control of microorganisms in hospital, medical and dental clinic, laboratory, food processing and food preparation environments. Failure to control microorganisms in these milieus may lead to the transmission of pathogens, illustrated by several outbreaks of salmonellosis and listeriosis resulting from the presence of pathogens in processed dairy products [3–5,9]. Effective disinfectants are available for use in the above and other areas, but the selection of a disinfectant by a user is dependent on many factors in addition to efficacy: information supplied by manufacturers and distributors, cost, ease of use, contact time required, and organic load encountered. Manufacturers and distributors generally supply information only on their own products, in large part due to the effort required to substantiate disinfectant activity for each product for each particular type of application, both for the customer and for registration purposes, which can differ from state to state and country to country. Papers in which many disinfectants are directly

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compared [6] are not common; even when available, the results presented may be difficult to interpret by individuals interested in applications of disinfectants other than those presented in a publication. Direct comparison of disinfectants in a relatively simple, non-specific test could assist users in the selection of a disinfectant.

Side-by-side evaluations of disinfectants could provide useful information on the procedures used to determine the activity of these compounds. Common procedures used in the U.S.A. for the determination of biocidal activity are based on the Official Methods of Analysis of the Association of Official Analytical Chemists (A.O.A.C.). [2]. Recently, alternatives to some of these methods have been proposed to improve the reliability and/or reproducibility of disinfectant assays [1,18], and concerns have been raised about the use of use dilution methodology [13].

In this paper the activity of 11 hard-surface disinfectants was compared using a procedure adapted from the germicidal and detergent sanitizer assay described by the A.O.A.C. [2]. These disinfectants were compared directly, and possible effects of the actual testing procedure on the observed activities were examined.

MATERIALS AND METHODS

Microorganisms and growth conditions

Pseudomonas aeruginosa ATCC 15442, Staphylococcus aureus ATCC 6538 and Saccharomyces cerevisiae ATCC 18824 were obtained from the American Type Culture Collection. Media used for these experiments were adapted from the synthetic broth described by the A.O.A.C. [2]. For *P. aeruginosa* and *S. aureus*, the culture medium contained (g/l): vitamin assay casamino acids (Difco), 10; Soytone (Difco), 3.0; glucose, 1.0; NaCl, 0.8; NH₄Cl, 1.0; KCl, 0.1; KH₂PO₄, 0.1; MgSO₄ · 7H₂O, 0.2; CaCl₂ · 2H₂O, 0.02; vitamin solution [22], 10 ml. The final pH was 7.3. This medium will support the growth of more fastidious bacteria, such as *Listeria monocytogenes*. Cultures were incubated at 37°C in shallow culture (30 ml medium/250 ml flask) either stationary or with gentle agitation. For *Sac. cerevisiae*, the level of glucose was increased to 20 g/l, the final pH was 6.0 and cultures were incubated at 30°C.

Disinfectants

The disinfectants tested are listed in Table 1. Disinfectants were from commercial sources and were selected as representative of products used in food/ dairy processing and/or clinical settings. For comparative testing of biocidal activity, each disinfectant was prepared according to label instructions. A recommended use level for hydrogen peroxide was estimated from the literature [8,12,21]. Total chlorine in some disinfectants was estimated using test kits (CHEMetrics K2505A, 0–250 ppm free and total chlorine; CHEMetrics K2505C, 0–5000 ppm free and total chlorine).

Test procedures

The basic test procedure for the comparative evaluations of disinfectants was adapted from the A.O.A.C. germicidal and detergent sanitizer test [2]. The methodology used to determine disinfectant activity was such that it would be based on established procedures, be technically easy to conduct, and provide for a severe test; i.e., conditions were used that would promote recovery of microorganisms from the disinfectant assay. All laboratory ware was new or chemically clean. Wherever possible, sterile/disposable materials were used. Synthetic hard water, 100 ppm hardness, was prepared as described by the A.O.A.C. [2], except that 0.23 g/l TES (N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid) was added to eliminate the need for pH adjustment after addition of the NaHCO₃ solution. The final pH of synthetic hard water should be between 7.6 and 8.0 [2]. The final pH of the hard water used in these experiments was 7.6; this was readily achieved by incorporating 1 mM TES and by adding NaH-CO₃ from a freshly prepared, filter-sterilized stock solution. It should be noted that aged solutions of NaHCO₃ can become very alkaline. All working solutions were buffered with 1 mM TES and contained calcium and magnesium (to 100 ppm water hardness). All test solutions were at a temperature of 22°C.

Disinfectants tested and recommended use

Disinfectant	Use concentration of active ingredients ^a (mg/l)	Contact time ^a (min)
Chlorine compound I	2 500 sodium hypochlorite ^b	10
Chlorine compound II	200 sodium hypochlorite ^e	2
Chlorine compound III	2 300 sodium chlorite ^d	1
Chlorine compound IV	500 chlorine dioxide ^e	1
Iodophor	280 α -(<i>p</i> -nonylphenyl)- ω -hydroxypoly(oxyethylene)-iodine complex ^f 250 phosphoric acid	10
Peroxide	30 000 hydrogen peroxide	60
Glutaraldehyde-phenol	1 200 glutaraldehyde	10
	4 400 phenol	
	1 500 sodium tetraborate	
	750 sodium phenate	
Acid glutaraldehyde ^g	5 000 glutaraldehyde	10
Quat	44 octyl decyl dimethyl ammonium chloride 22 didecyl dimethyl ammonium chloride	1
	22 dioctyl dimethyl ammonium chloride	
	59 alkyl (C ₁₄ , 50%; C ₁₂ , 40%; C ₁₆ , 10%) dimethyl benzyl ammonium chloride	
Acidified quat ^h	44 octyl decyl dimethyl ammonium chloride	1
	22 didecyl dimethyl ammonium chloride	
	22 dioctyl dimethyl ammonium chloride	
	59 alkyl (C ₁₄ , 50%; C ₁₂ , 40%; C ₁₆ , 10%) dimethyl benzyl ammonium chloride	
Phenolic	520 o-phenylphenol	10
	250 o-benzyl-p-chlorophenol	

^a Recommended use concentration of active ingredients and recommended contact time are from label instructions for each disinfectant, except for hydrogen peroxide. A recommended use concentration and contact time for hydrogen peroxide was estimated from the literature [8,12,21].

^b 1400 mg/l total chlorine (estimated).

^c 140 mg/l total chlorine (estimated).

^d 590 mg/l total chlorine (estimated). After acid activation according to label instructions, the active ingredient in chlorine compound III is chlorine dioxide.

^e 120 mg/l total chlorine (estimated).

^f 27 mg/l titratable iodine.

^g Contains a non-ionic surfactant.

^h Contains an undisclosed amount of phosphoric acid.

Freshly prepared disinfectants were added to sterile, disposable 250 ml polycarbonate flasks containing sterile synthetic hard water for a final volume of 50 ml. Each disinfectant was prepared according to label instructions and added to a flask at concentrations based on the recommended use level $(\frac{1}{4} \times , \frac{1}{2} \times , 1 \times , 2 \times , \text{etc.})$.

Each test was initiated by the addition of a test organism: 0.5 ml of a 24-h culture of *P. aeruginosa*; 1.0 ml of a 24-h culture of *S. aureus*; 5.0 ml of a 48-h

culture of *Sac. cerevisiae*. Tests were initiated by the addition of a test organism rather than disinfectant because some disinfectants required more than one addition to constitute. Broth cultures were used for inocula rather than resuspended agar cultures to facilitate handling of the microorganisms, an important consideration if testing against pathogens. The addition of culture medium meant that a small organic load was added to each test. Viable cell counts of controls were determined by inoculating test

flasks containing 50 ml of synthetic hard water and sampling after 60 s, 1.0 ml of sample being placed into 4.0 ml of a neutralizing solution. For disinfectant tests, at 30 and 60 s after inoculation, 1.0 ml of sample from a test flask was added to 4.0 ml of a neutralizing solution. For the halogen-based disinfectants, the neutralizing solution contained $Na_2S_2O_3$ (2.0 g/l synthetic hard water, 100 ppm hardness). For the glutaraldehyde-based disinfectants and hydrogen peroxide, the neutralizing solution contained NaHSO₃ (5.0 g/l synthetic hard water). For P. aeruginosa and hydrogen peroxide, synthetic hard water alone was used. For the surface-active disinfectants, the neutralizing solution contained polyoxyethylene sorbitan mono-oleate (Tween 80) (5.0 g/l synthetic hard water). All neutralizing solutions were freshly prepared and filtersterilized.

Tests were conducted by two people working in tandem so that serial dilutions for viable counts were initiated within seconds after each time point (when a test sample was added to the neutralizer). Each sample was serially diluted for viable cell counts in the original culture medium containing 10 g/l purified agar (Difco). Enumeration plates of *P. aeruginosa* and *S. aureus* were incubated at 37°C for 48 h. Enumeration plates of *Sac. cerevisiae* were incubated at 30°C for 72 h. Each disinfectant concentration was tested in duplicate and at each time point duplicate samples were plated for enumeration.

Materials

All of the common chemicals were of reagent grade or better. The disinfectants were obtained from both manufacturers and distributors of these products. Hydrogen peroxide (31.0% assay) was obtained from Fisher Scientific. Media components were obtained from Difco Laboratories.

RESULTS AND DISCUSSION

The activity of each disinfectant against each test organism is given in Tables 2, 3 and 4; results are presented as the viable cell count observed in each test. The viable cell counts are an average of the duplicate serial dilution plating for each test. When viable counts were similar for duplicates of a test condition, the results were averaged. A summary of the test results is presented in Table 5; only if $a \ge 5$ log reduction in viable counts was observed in both tests for a disinfectant concentration was that concentration given in Table 5. The prime purpose of this experimental series was to compare activity of disinfectants directly in a 1-min time frame. Discussion of the results for different classes of disinfectants follows.

Halogens

The two disinfectants containing sodium hypochlorite, chlorine compounds I and II, performed similarly in these tests. Both of the eubacterial species and the eukaryote were affected by similar levels of a particular halogen-based disinfectant, with the exception of chlorine compound III (sodium chlorite).

At label-recommended use concentrations, chlorine compounds I, III and IV performed well against the test organisms. For disinfection in the clinical environment, use concentrations of 1000– 5000 mg/l free chlorine for chlorine compounds and 70–150 mg/l for iodophors should be used [8]. On the basis of the results presented in Tables 2–5, the halogen-based disinfectants tested in this study would all perform satisfactorily in a clinical environment if used at those concentrations.

On the basis of results presented in the literature [7], it had been anticipated that the disinfectants containing sodium hypochlorite would have had greater biocidal activity in this assay. Two factors influencing the activity of hypochlorite are organic load and pH. The pH of the synthetic hard water used in these studies was 7.6. The addition of cells in culture medium tended to lower the pH and provided additional buffering capacity to the test solution (Table 6). If cells were pelleted and resuspended in synthetic hard water, some of this buffering capacity was lost (Table 6). As shown in Table 6, sodium hypochlorite was significantly more active (> 5 log reduction in 60 s with 130 mg/l vs. 1000 mg/l) against cells pelleted and resuspended than cells

Activity of disinfectants against P. aeruginosa

Disinfectant ^a	Active ingredient(s) (mg/l) ^a	Viable cell count/ml		
		30 s	60 s	control
Chlorine compound I	1 000	$<2 \times 10^{\circ}$	$<2 \times 10^{\circ}$	2×10^{8}
-		8×10^4	$<2 \times 10^{\circ}$	2×10^{8}
	510	$> 1 \times 10^7$	$>1 \times 10^{7}$	3×10^8
Chlorine compound II	820	$< 2 \times 10^{0}$	$<2 \times 10^{0}$	3×10^{8}
		1×10^{3}	$<2 \times 10^{0}$	3×10^{8}
	420	3×10^{2}	$<2 \times 10^{0}$	1×10^{8}
		6×10^{6}	3×10^{6}	1×10^{8}
Chlorine compound III	310	$< 1 \times 10^{0}$	$<1 \times 10^{0}$	1×10^{8}
-	160	9×10^{6}	7×10^{5}	1×10^{8}
		1×10^{6}	8×10^{1}	1×10^8
Chlorine compound IV	48	2×10^4	$<2 \times 10^{0}$	2×10^8
•		2×10^2	2×10^{1}	2×10^{8}
Iodophor	440	4×10^{1}	1×10^{1}	1×10^8
-		$5 \times 10^{\circ}$	$2 \times 10^{\circ}$	1×10^{8}
	210	3×10^{5}	2×10^4	1×10^8
		2×10^4	2×10^3	1×10^{8}
Peroxide	36 000	$< 1 \times 10^{0}$	$< 1 \times 10^{0}$	2×10^8
	19 000	2×10^{3}	$<2 \times 10^{0}$	2×10^{8}
		2×10^4	6×10^3	2×10^8
Glutaraldehyde-phenol	2 300	$< 2 \times 10^{0}$	$<2 \times 10^{\circ}$	3×10^{8}
		4×10^{3}	$<2 \times 10^{0}$	3×10^{8}
	1 200	2×10^{6}	2×10^4	1×10^{8}
Acid glutaraldehyde	6 600	3×10^4	$<1 \times 10^{0}$	2×10^{8}
	3 900	6×10^{6}	7×10^5	8×10^{7}
Quat	580	5×10^{1}	5×10^{1}	2×10^{8}
		$5 \times 10^{\circ}$	$2 \times 10^{\circ}$	2×10^{8}
	290	7×10^2	5×10^{2}	1×10^{8}
		8×10^3	8×10^3	1×10^{8}
Acidified quat	150	$< 1 \times 10^{\circ}$	$<1 \times 10^{0}$	2×10^{8}
	73	7×10^{4}	2×10^{4}	3×10^{8}
		4×10^3	2×10^2	3×10^{8}
Phenolic	1 500	2×10^4	$5 \times 10^{\circ}$	2×10^{8}
		5×10^2	$< 2 \times 10^{0}$	2×10^{8}
	760	$>1 \times 10^{7}$	2×10^{6}	3×10^{8}

* Disinfectants and active ingredients are given in Table 1.

added in the original culture broth, even though the pH was lower in tests when medium was added. A possible reason for the increase in observed biocidal activity, in addition to the removal of an organic load, might be non-specific lowering of resistance to a disinfectant as a result of the washing procedure. This was observed in a comparison of other disinfectant tests [17] and was observed in determination

Activity of disinfectants against S. aureus

Disinfectant ^a	Active ingredient(s) (mg/l)	Viable cell count/ml		
		30 s	60 s	control
Chlorine compound I	1 000 500	$<1 \times 10^{0}$ >1 × 10 ⁷	$<1 \times 10^{0}$ >1 × 10 ⁷	3×10^7 2×10^8
Chlorine compound II	820 410	$<1 \times 10^{0}$ >1 × 10 ⁷	$<1 \times 10^{0}$ >1 × 10 ⁷	$\begin{array}{rrr} 2 \ \times \ 10^8 \\ 4 \ \times \ 10^7 \end{array}$
Chlorine compound III	1 300 620	5×10^{0} 7×10^{4} 9×10^{3}	$<1 \times 10^{0}$ 8 × 10 ² 3 × 10 ¹	1×10^{7} 3×10^{7} 3×10^{7}
Chlorine compound IV	93 48	4×10^{0} 5×10^{3} 3×10^{3}	$<1 \times 10^{0}$ 2 × 10 ³ 1 × 10 ¹	1×10^7 1×10^7 1×10^7
Iodophor	440 210	1×10^{1} 1×10^{3} 9×10^{4}	$<2 \times 10^{0}$ $<2 \times 10^{0}$ 5×10^{3}	2×10^{8} 2×10^{8} 4×10^{7}
Peroxide	68 000 36 000	2×10^{3} <1 × 10 ⁰ 5 × 10 ⁴	4×10^{3} <1 × 10 ⁰ 4 × 10 ⁴	4×10^{7} 3×10^{7} 3×10^{7}
Glutaraldehyde-phenol	1 200 600	$ \begin{array}{rrr} <1 \ \times \ 10^{0} \\ 6 \ \times \ 10^{4} \end{array} $	$ \begin{array}{rrr} <1 \ \times \ 10^{0} \\ 8 \ \times \ 10^{3} \end{array} $	$\begin{array}{l} 2 \times 10^7 \\ 4 \times 10^7 \end{array}$
Acid glutaraldehyde	2 200	1×10^{1} 4×10^{2} 0×10^{4}	$<2 \times 10^{0}$ $<2 \times 10^{0}$ $^{9} \times 10^{3}$	4×10^{7} 4×10^{7} 2×10^{7}
Quat	140	4×10^{3} 2×10^{2}	$< 2 \times 10^{\circ}$ $< 2 \times 10^{\circ}$ $< 2 \times 10^{\circ}$	$\frac{2 \times 10^6}{3 \times 10^6}$
	72	6×10^4	6×10^2	3×10^7
Acidified quat	1 200 580	$\begin{array}{r} 4 \times 10^{4} \\ 8 \times 10^{4} \end{array}$	$ \begin{array}{rrr} <1 \ \times \ 10^{0} \\ 3 \ \times \ 10^{4} \end{array} $	$\begin{array}{r} 3 \times 10^7 \\ 3 \times 10^6 \end{array}$
Phenolic	380	8×10^{1} < 2 × 10 ⁰ 3 × 10 ⁴	$<2 \times 10^{0}$ $<2 \times 10^{0}$ 5×10^{2}	3×10^{6} 3×10^{6} 3×10^{7}
	170	2×10^4	$2 \times 10^{-10^{-10^{-10^{-10^{-10^{-10^{-10^{-$	3×10^{7} 3×10^{7}

^a Disinfectants and active ingredients are given in Table 1.

of the activity of chlorine compound IV, chlorine dioxide, against *L. monocytogenes* (R.S. Tanner, unpublished results). To study the effect of organic load due to medium addition, casamino acids, Soytone or glucose was eliminated from the medium and the activity of chlorine compound I against *S. aureus* retested. The activities were essentially identical to that for cell addition in complete medium (data not shown). If lowering of the activity of sodium hypochlorite was due just to the organic load, one must explain the lack of relative response to different levels of medium addition (0.5 ml of culture for *P. aeruginosa* vs. 5.0 ml of culture for *Sac. cerevisiae*) and the probable lack of effect of medi-

Activity of disinfectants against Sac. cerevisiae

Disinfectant ^a	Active	Viable cell count/ml		
	ingredient(s) (mg/l) ^a	30 s	60 s	control
Chlorine compound I	1 000	<1 × 10 ⁰	<1 × 10 ⁰	2×10^{6}
	520	6×10^{2}	5×10^{1}	4×10^{6}
		6×10^3	2×10^2	4×10^{6}
Chlorine compound II	1 600	$<1 \times 10^{0}$	$<1 \times 10^{0}$	4×10^{6}
	830	7×10^2	6×10^{1}	2×10^{6}
		$<2 \times 10^{0}$	$< 2 \times 10^{\circ}$	2×10^{6}
Chlorine compound III	640	$2 \times 10^{\circ}$	$< 1 \times 10^{0}$	4×10^{6}
	330	3×10^{2}	1×10^2	3×10^{5}
		6×10^{1}	$< 2 \times 10^{\circ}$	3×10^{5}
Chlorine compound IV	95	$1 \times 10^{\circ}$	$<1 \times 10^{0}$	4×10^{6}
-	49	2×10^{3}	1×10^{3}	2×10^{6}
		4×10^2	3×10^{2}	2×10^{6}
Iodophor	450	$< 2 \times 10^{0}$	<2 × 10"	1×10^{6}
-		1×10^{2}	$<2 \times 10^{0}$	1×10^{6}
	220	2×10^{5}	3×10^{5}	2×10^{6}
		6×10^4	8×10^3	2×10^6
Peroxide	270 000	$< 1 \times 10^{0}$	$<1 \times 10^{0}$	4×10^{5}
	140 000	2×10^{5}	9×10^4	3×10^5
Glutaraldehyde-phenol	620	4×10^3	$<2 \times 10^{0}$	2×10^{6}
		8×10^4	2×10^{1}	2×10^{6}
	320	2×10^4	1×10^{3}	3×10^{5}
Acid glutaraldehyde	18 000	$<2 \times 10^{0}$	$<2 \times 10^{0}$	4×10^{5}
		1×10^{1}	$<2 \times 10^{\circ}$	4×10^{5}
	12 000	4×10^{3}	6×10^2	2×10^{5}
		2×10^3	4×10^{1}	2×10^5
Quat	74	1×10^2	$<2 \times 10^{0}$	4×10^5
		3×10^{3}	$<2 \times 10^{0}$	4×10^5
	37	3×10^4	4×10^3	1×10^{6}
Acidified quat	300	2×10^{3}	$<1 \times 10^{0}$	3×10^{5}
	150	1×10^{3}	8×10^{1}	4×10^5
		3×10^3	$<2 \times 10^{0}$	4×10^5
Phenolic	190	$<1 \times 10^{0}$	$<1 \times 10^{0}$	4×10^5
	87	2×10^5	2×10^3	1×10^{6}
		1×10^{5}	2×10^2	1×10^{6}

^a Disinfectants and active ingredients are given in Table 1.

um addition on the activity of chlorine compounds III and IV and the iodophor. This was beyond the scope of the present study. Chlorine compounds I and II are alkaline, whereas the disinfectant tests were acidified by the addition of chlorine compound III, chlorine compound IV and the iodophor due to the presence of lactic acid, citric acid and phosphoric acid, respectively, in these disinfectants. 152

Table 5

Disinfectant concentration tested resulting in a 99.999% reduction in viable cell counts after a 60-s exposure

Disinfectant ^a	Test organism				
(ing/1)	P. aeruginosa	S. aureus	Sac. cerevisiae		
Chlorine compound I	1 000	1 000	1 000		
Chlorine compound II	820	820	1 600		
Chlorine compound III	310	1 300	640		
Chlorine compound IV	48	93	95		
Iodophor	440	440	450		
Peroxide	36 000	68 000	270 000		
Glutaraldehyde-phenol	2 300	1 200	620		
Acid glutaraldehyde	6 600	2 200	18 000		
Quat	580	140	74		
Acidified quat	150	1 200	300		
Phenolic	1 500	380	190		

^a Disinfectants and active ingredients are given in Table 1.

As reported elsewhere [10], disinfectants containing chlorine dioxide (chlorine compounds III and IV) had greater biocidal activity than hypochlorite.

Peroxide

High concentrations of hydrogen peroxide (27% for *S. cerevisiae*) were required to achieve disinfecting reductions in viable counts in 60 s against the test organisms, as shown in Tables 2–5. The results are even more significant in light of the fact that the best neutralizer, catalase [19], was not used in these assays. However, the results are in good agreement with the observations summarized by Turner [21], including the kinetic curve for the activity of 3% hydrogen peroxide against *P. aeruginosa* and *S. aureus*, and the fact that, stated in *D* values, *P. aeruginosa* was more sensitive to hydrogen peroxide than *S. aureus*, which in turn was more sensitive than fungi.

Glutaraldehyde

In agreement with the observations summarized by Scott and Gorman [20], the acid glutaraldehyde did not have as much biocidal activity as the (alkaline) glutaraldehyde-phenol disinfectant, and *P. aeruginosa* was more resistant than *S. aureus* to the

Table 6

Effect of culture medium on the disinfectant activity of sodium hypochlorite

Condition	NaOCl ^a	рН	Viable cell count/ml	
	(mg/1)		60 s	control
Cells in culture medium ^b Cells resuspended in synthetic hard water ^e	130 130	7.1 9.1	1×10^{6} $< 2 \times 10^{0}$	$\begin{array}{c} 1 \ \times \ 10^6 \\ 1 \ \times \ 10^6 \end{array}$

^a Chlorine compound I in Table 1.

^b 1.0 ml of a 24-h culture of *S. aureus* added to 49 ml of synthetic hard water for test. Medium described in Materials and Methods.

° A 24-h culture of *S. aureus* was pelleted by centrifugation (5000 \times g, 10 min, 22°C). Cell pellet was resuspended in synthetic hard water, 100 ppm hardness, and cells added to a test flask.

biocidal action of glutaraldehyde. Phenol probably contributed significantly to the activity of the glutaraldehyde-phenol disinfectant against *Sac. cerevisiae*. It should be noted that the results presented in Tables 2–5 are for activities in a 1-min assay and that 10-min contact times are normally recommended for glutaraldehyde-based disinfectants (Table 1).

Quaternary ammonium compounds

Quats are generally considered fast-acting disinfectants, active at low concentrations [15]. In agreement with the observations summarized by Petrocci [15], *P. aeruginosa* was less sensitive than *S. aureus* to the quat disinfectant, and, in general, acidification lowered the activity of the quat. The acidified quat disinfectant was more active against *P. aeruginosa*, a result predicted based on the acid sensitivity of this microorganism [14]. *Sac. cerevisiae* was very sensitive to the quat disinfectant in this assay.

Phenolic compounds

In agreement with the observations summarized by Prindle [16], *P. aeruginosa* was more resistant than *S. aureus* to the activity of the phenolic disinfectant. As with the glutaraldehyde-based disinfectants, the contact time normally recommended for phenolic disinfectants is 10 min.

In summary, the results presented permit the direct comparison of 11 disinfectants in a 60-s speedof-kill assay. This is not always an easy evaluation to make from the literature and product information, where results may be presented as D values or phenol coefficients, and disinfectant concentrations given as ppm, dilution ratios or percentages, sometimes even in the same data table [11]. Potential users of disinfectants should evaluate biocidal test results in the context of intended use. Here, where activities were measured over a 1-min time frame. the results presented in this paper should be useful for preliminary review of disinfectants for an application such as food or dairy processing, where contact times for a disinfectant might be very short. The results presented here suggest that, in general, halogen-based disinfectants would perform well in such applications. The chlorine compound contain-

ing chlorine dioxide was the most active in this assay, based on mg/l active ingredient. One of the sodium hypochlorite disinfectants and the sodium chlorite disinfectant also performed well in this assay when effective disinfecting concentrations of active ingredients (Table 5) are compared to recommended use levels (Table 1). The results presented here might not be as useful for an application such as a disinfectant soak in a clinical setting where contact times are generally at least 10 min. Biocidal activity in a suspension test is one of several factors (organic load, corrosiveness, etc.) that must be considered for actual disinfectant applications. Considering the possible need to reexamine current methodologies used for the evaluation of disinfectants [1,13,18], the results presented here may be useful for consideration of suspension testing as an important aspect of the evaluation of disinfectants.

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REFERENCES

- Ascenzi, J.M., R.J. Ezzell and T.M. Wendt. 1987. A more accurate method for measurement of tuberculocidal activity of disinfectants. Appl. Environ. Microbiol. 53: 2189–2192.
- 2 Association of Official Analytical Chemists. 1980. Disinfectants. In: Official Methods of Analysis, 13th ed. (Horowitz, W., ed.), pp. 56–68, Association of Official Analytical Chemists, Washington, DC.
- 3 Centers for Disease Control. 1984. Salmonellosis associated with cheese consumption Canada. MMWR 33: 387.
- 4 Centers for Disease Control. 1985. Update: Milk-borne salmonellosis Illinois. MMWR 34: 215–216.
- 5 Centers for Disease Control. 1985. Listeriosis outbreak associated with Mexican-style cheese – California. MMWR 34: 357–359.
- 6 Dwire, K.M. and J.F. James. 1982. Comparative testing and evaluation of germicidal solutions used for the sterilization

or disinfection of medical and dental instruments and equipment. ADM Lab. J. 12: 1-8.

- 7 Dychdala, G.R. 1983. Chlorine and chlorine compounds. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 157–182, Lea & Febiger, Philadelphia, PA.
- 8 Favero, M.S. 1983. Chemical disinfection of medical and surgical materials. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 469–492, Lea & Febiger, Philadelphia, PA.
- 9 Fleming, D.W., S.L. Cochi, K.L. MacDonald, J. Brondum, P.S. Hayes, B.D. Plikaytis, M.B. Holmes, A. Audurier, C.V. Broome and A.L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. 312: 404–407.
- 10 Geldreich, E.E. 1986. Potable water: new direction in microbial regulations. ASM News 52: 530–534.
- 11 Gottardi, W. 1983. Iodine and iodine compounds. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 183–196, Lea & Febiger, Philadelphia, PA.
- 12 Jones, R. 1987. Sanitizing your water system: a critical step in your process. Microfiltration News (Gelman Sciences) 7: 4.
- 13 Meyers, T. 1988. Failing the test: germicides or use dilution methodology? ASM News 54: 19–21.
- 14 Morton, H.E. 1983. *Pseudomonas*. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 401– 413, Lea & Febiger, Philadelphia, PA.
- 15 Petrocci, A.N. 1983. Surface-active agents: quaternary am-

monium compounds. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 309–329, Lea & Febiger, Philadelphia, PA.

- 16 Prindle, R.F. 1983. Phenolic compounds. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 197–224, Lea & Febiger, Philadelphia, PA.
- 17 Reybrouck, G. 1980. A comparison of the quantitative suspension tests for the assessment of disinfectants. Zent.bl. Bakteriol. Hyg. Abt. 1 Orig. B 170: 449-456.
- 18 Robison, R.A., H.L. Bodily, D.F. Robinson and R.P. Christensen. 1988. A suspension method to determine reuse life of chemical disinfectants during clinical use. Appl. Environ. Microbiol. 54: 158–164.
- 19 Russell, A.D. 1983. Principles of antimicrobial activity. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 717–750, Lea & Febiger, Philadelphia, PA.
- 20 Scott, E.M. and S.P. Gorman. 1983. Sterilization with glutaraldehyde. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 65–88, Lea & Febiger, Philadelphia, PA.
- 21 Turner, F.J. 1983. Hydrogen peroxide and other oxidant disinfectants. In: Disinfection, Sterilization and Preservation, 3rd ed. (Block, S.S., ed.), pp. 240–250, Lea & Febiger, Philadelphia, PA.
- 22 Wolin, E.A., M.J. Wolin and R.S. Wolfe. 1963. Formation of methane by bacterial extracts. J. Biol Chem. 238: 2882–2886.