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COMPARATIVE STUDY OF BACTERICIDAL ACTIVITIES OF SIX DIFFERENT DISINFECTANTS

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ABSTRACT

Bactericidal activities of six commonly used disinfectants against seven different species of clinically isolated bacteria, mainly glucose-nonfermentative Gram-negative bacilli, were compared. In terms of mean values of the minimum bactericidal concentration (MBC), benzethonium chloride showed the highest efficacy, followed in order by chlorhexidine gluconate, alkyldiaminoethylglycine hydrochloride, glutaraldehyde, povidone-iodine, and phenol. However, mean MBCs of benzethonium chloride, alkyldiaminoethylglycine hydrochloride and chlorhexidine gluconate against individual species covered a far wider range from species to species compared with those of the other three disinfectants. In addition, bactericidal activities of the above-mentioned three disinfectants against *Pseudomonas cepacia* and *Achromobacter xylosoxidans* ranged more widely from strain to strain than against other species. When the minimum concentrations of individual disinfectants recommended for hospital use by the manufacturers were used, chlorhexidine gluconate (0.02%), alkyldiaminoethylglycine hydrochloride (0.1%), benzethonium chloride (0.1%) and povidone-iodine (0.75%) were not bactericidal to definite numbers of bacterial strains tested. Among them, chlorhexidine gluconate at the concentration recommended was ineffective against many strains of all bacterial species tested, especially against strains of glucose-nonfermentative Gram-negative bacilli except for *Pseudomonas aeruginosa*. Alkyldiaminoethylglycine hydrochloride was also remarkably ineffective against *Staphylococcus aureus* and *P. cepacia*. Among all the species tested, *P. cepacia* showed the strongest resistance to the disinfectants tested at the minimum concentrations of individual disinfectants recommended for hospital use by the manufacturers.

Key words: Disinfectant, bactericidal activity

INTRODUCTION

Knowledge about infections and modes of sterilization has become more and more important with the increase in the prevalence of and changes in the type of nosocomial infection. Several previous investigators have reported on the rapidity of bactericidal activity and the minimum inhibitory concentration (MIC) for a variety of disinfectants.[1-5] However, there have been few reports to date on the simultaneous comparison of the bactericidal activity of various disinfectants exposed for a finite time using the same strains of bacterial species.

The pattern of nosocomial infections, historically dominated by Gram-positive *Staphylococcus aureus* in the 1950's, has shifted to a predominance of infections due to the

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glucose-fermentative Gram-negative *Enterobacteriaceae* (i.e., *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Serratia* and *Proteus*) and glucose-nonfermentative Gram-negative *Pseudomonas aeruginosa*. Recently, glucose-nonfermentative Gram-negative bacilli other than *Pseudomonas aeruginosa* have emerged as important opportunistic pathogens.[6-9] In addition, there were several reports on contamination of stored disinfectant solution by glucose-nonfermentative Gram-negative bacilli which resulted in outbreaks of nosocomial infections.[10-19]

In the work reported here, we compared the bactericidal activity of six commonly used disinfectants against seven different species of bacteria, mainly glucose-nonfermentative Gram-negative rods. All the bacteria tested were isolated from patients in our hospital.

In order to neutralize the activity of the disinfectant carried over from the bacteria/disinfectant mixture into the recovery medium most of the previous studies on disinfectants used chemical inactivators. However, there is no assurance that the disinfectant carried over into the recovery medium will be fully neutralized by the inactivator, and some inactivators themselves have an inhibitory effect on the growth of microorganisms.[20,21] Since it is almost impossible to compute and add just the proper amount of an inactivator that will equally neutralize the carried-over disinfectant in each recovery medium, we devised a new control method to avoid the use of any inactivators and to ascertain that the disinfectant carried over from the bacteria/disinfectant mixture does not inhibit the growth of bacteria in the recovery medium.

MATERIALS AND METHODS

Preparation of the inoculum

Twenty strains each of seven bacterial species of clinical isolates were used: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas maltophilia*, *Achromobacter xylosoxidans*, and *Acinetobacter anitratus*. Two days before the test, the organisms to be tested were inoculated to modified Mueller-Hinton agar (Nissui Pharmaceutical Manufacturing Co., Tokyo, Japan) and incubated for 24h at 37°C. Next they were inoculated into 100ml of heart infusion broth (Tanabe Pharmaceutical Manufacturing Co., Osaka, Japan), incubated for 24h at 37°C, and then well shaken and used as the inoculum for the test.

Viable organisms in the inoculum were counted using nutrient agar plate. Calculated inocula per milliliter were as follows: *S. aureus*, $(2.59 \pm 1.42) \times 10^8$; *E. coli*, $(3.15 \pm 1.07) \times 10^8$; *P. aeruginosa*, $(3.88 \pm 1.66) \times 10^8$; *P. cepacia*, $(9.21 \pm 5.81) \times 10^7$; *P. maltophilia*, $(1.42 \pm 0.62) \times 10^8$; *A. xylosoxidans*, $(2.65 \pm 1.31) \times 10^7$; *A. anitratus*, $(1.65 \pm 1.28) \times 10^8$.

Praparation of disinfectant dilution

Six different disinfectants were used: benzethonium chloride, chlorhexidine gluconate, alkyldiaminoethylglycine hydrochloride, glutaraldehyde, povidone-iodine, and phenol. Serial twofold dilutions (3ml) of each disinfectant solution were prepared in the test tubes using distilled water as diluent.

The test method

The test method is shown in Figure 1. The test for the bactericidal activities of the six different disinfectants were performed at room temperature using the same strains of bacteria on the same day. Heart infusion recovery broth tubes were prepared with 5ml aliquots and arranged in two series of 3 tubes each, the one series for 10-min exposure and the other for 20-

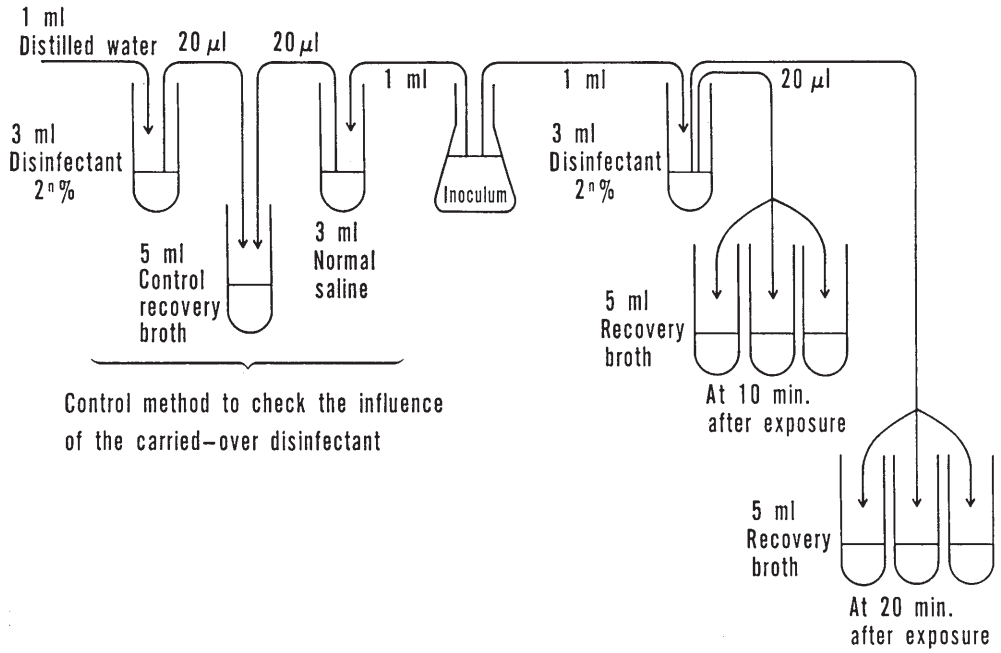

FIG. 1. Diagrammatic representation of the test method

min exposure. One ml of the inoculum was added to each of the serial twofold dilutions (3ml) of each disinfectant and the contents were mixed by shaking. After the 10- and 20-min incubation periods, 20μl of the sample was sequentially withdrawn and added to the corresponding three tubes of the recovery broth. All of the recovery broth tubes were incubated for 48h at 37°C, and then examined for the lowest concentration of disinfectant which showed no growth of bacteria in at least two out of the three recovery broths, i.e., the minimum bactericidal concentration (MBC) of the tested disinfectant against the tested strain after the respective contact time of 10 and 20 min.

In order to rule out the possibility that the absence of bacterial growth seen in the recovery broth was due to the effect of the disinfectant which was carried over together with bacteria from the mixture into the recovery broth, a control method was devised and simultaneously carried out. One ml of the inoculum used in the test was added to 3ml of normal saline, and 1ml of distilled water was added to each of the serial twofold dilutions (3ml) of the disinfectants. Twenty μl each of the inoculum and the disinfectant were added to 5ml of a control recovery broth of the same composition as used in the test, and incubated for 48h at 37°C. When there is no bacterial growth in the recovery broth into which the sample treated with a certain concentration of a disinfectant was added and there is no inhibition of bacterial growth in the control recovery broth, the absence of growth seen in the test can be said to be due to the bactericidal action of the concentration of the tested agent obtained by 10- and 20-min exposure. In contrast, when inhibition of bacterial growth occurs in the control recovery broth, it can not be determined whether the absence of bacterial growth in the recovery broth in the test, results from killing of bacteria by the disinfectant during 10- or 20-min exposure or from the action of the disinfectant carried over into the recovery broth. In most cases shown

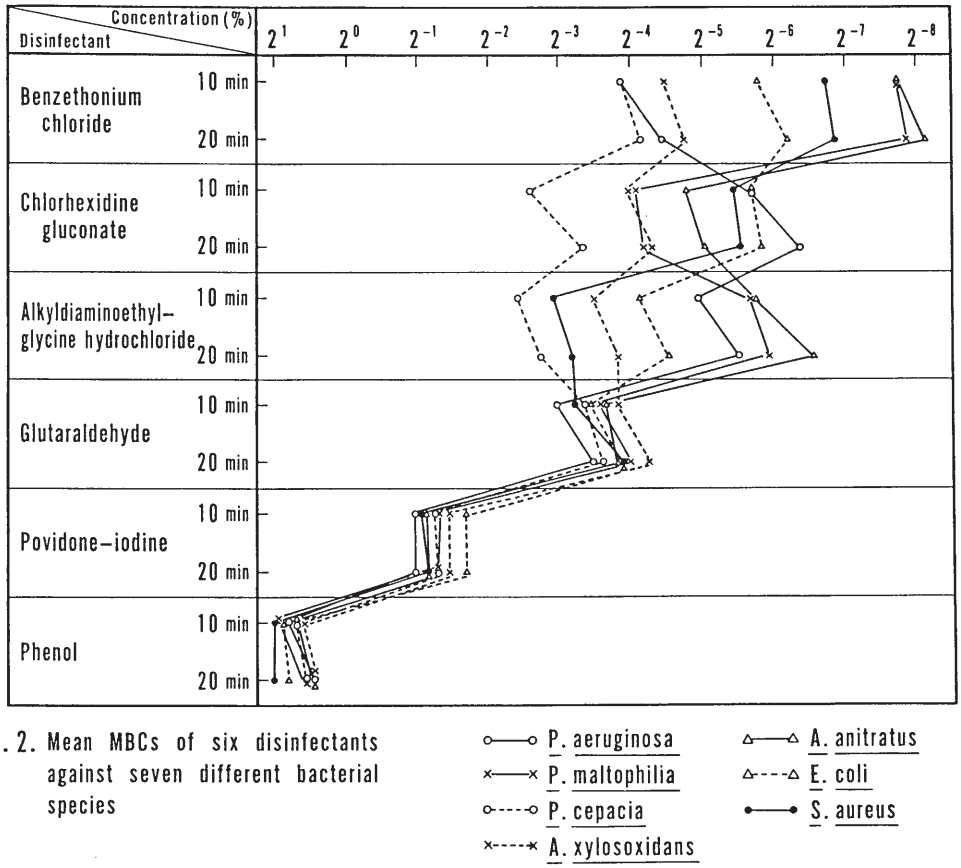

FIG. 2. Mean MBCs of six disinfectants against seven different bacterial species

in the present study, it could be confirmed by the control method that the absence of bacterial growth in the test was not due to the residual effect of the disinfectant carried over into the recovery broth but due to the bactericidal action of the disinfectants during 10- or 20-min exposure.

RESULTS

The MBCs of the six different disinfectants against the seven bacterial species are shown in Table 1-7, and their mean MBCs against each of the bacterial species tested are shown in Figure 2. With respect to 2 out of 20 strains of *S. aureus* in the test for alkyldiaminoethylglycine hydrochloride and to 10 out of 20 strains of *S. aureus* in the test for chlorhexidine gluconate, inhibition of growth occurred in the control recovery broth into which the disinfectants were carried over from the same, or lower, concentrations as the minimum concentration of the disinfectants capable of exhibiting bactericidal action. Therefore, as far as these strains of *S. aureus* are concerned, the inhibitory effect of the two disinfectants carried over into the recovery broth from the inoculum/disinfectants mixture could not be completely denied and the efficacy of these two disinfectants on *S. aureus* were expressed as MIC in place of MBC (Table 1, asterisks).

Table 1. MBCs of six different disinfectants against *S. aureus*§

Disinfectant	Concentration (%)	2^{1}	2^{0}	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	MEAN ± S.D.
Benzethonium chloride	10min								5	15	$2^{-6.75\pm0.44}$
	20min								3	17	$2^{-6.85\pm0.37}$
Chlorhexidine gluconate	10min						1	9	10		$2^{-5.45\pm0.60}$ *
	20min						1	7	12		$2^{-5.55\pm0.60}$ *
Alkyldiaminoethyl-glycine hydrochloride	10min				1	19					$2^{-2.95\pm0.22}$ *
	20min				1	14	5				$2^{-3.20\pm0.52}$ *
Glutaraldehyde‖	10min				3	9	8				$2^{-3.25\pm0.72}$
	20min					1	19				$2^{-3.95\pm0.22}$
Povidone-iodine	10min		2	15	3						$2^{-1.05\pm0.51}$
	20min			17	3						$2^{-1.15\pm0.37}$
Phenol	10min	20									$2^{1.00\pm0.00}$
	20min	20									$2^{1.00\pm0.00}$

§ Twenty strains were tested for each disinfectant. Figures in the table represent the number of strains showing the respective MBCs. The number of bacteria inoculated into each tube was $(2.59 \pm 1.42) \times 10^{8}$.

‖ Significant difference between values at 10 and 20 min. $p<0.05$.

* Mean MIC ± S.D.

Table 2. MBCs of six different disinfectants against *E. coli*§

Disinfectant	Concentration (%)	2^{1}	2^{0}	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	MEAN ± S.D.
Benzethonium chloride	10min						1	5	11	3	$2^{-5.80\pm0.77}$
	20min							4	8	8	$2^{-6.20\pm0.77}$
Chlorhexidine gluconate	10min						3	4	9	4	$2^{-5.70\pm0.98}$
	20min						2	5	7	6	$2^{-5.85\pm0.99}$
Alkyldiaminoethyl-glycine hydrochloride	10min				2	1	9	8			$2^{-4.15\pm0.93}$
	20min				1		4	13	1		$2^{-4.55\pm1.00}$
Glutaraldehyde‖	10min					11	9				$2^{-3.45\pm0.51}$
	20min					1	19				$2^{-3.95\pm0.22}$
Povidone-iodine	10min			6	14						$2^{-1.70\pm0.47}$
	20min			6	14						$2^{-1.70\pm0.47}$
Phenol	10min	18	2								$2^{0.90\pm0.31}$
	20min	16	4								$2^{0.80\pm0.41}$

§ Twenty strains were tested for each disinfectant. Figures in the table represent the number of strains showing the respective MBCs. The number of bacteria inoculated into each tube was $(3.15 \pm 1.07) \times 10^{8}$.

‖ Significant difference between values at 10 and 20 min. $p<0.05$.

Table 3. MBCs of six different disinfectants against *P. aeruginosa*§

Disinfectant	Concentration (%)	2^{1}	2^{0}	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}	MEAN ± S.D.
Benzethonium‖ chloride	10min					7	8	5				$2^{-3.90\pm0.79}$
	20min					2	8	9	1			$2^{-4.45\pm0.76}$
Chlorhexidine‖ gluconate	10min					1		8	7	3	1	$2^{-5.70\pm1.08}$
	20min						1	2	7	8	2	$2^{-6.40\pm0.99}$
Alkyldiaminoethyl-glycine hydrochloride	10min					3	4	5	7	1		$2^{-4.95\pm1.19}$
	20min					1	1	6	10	2		$2^{-5.55\pm0.94}$
Glutaraldehyde‖	10min				3	14	3					$2^{-3.00\pm0.56}$
	20min				1	8	11					$2^{-3.50\pm0.61}$
Povidone-iodine	10min		1	19								$2^{-0.95\pm0.22}$
	20min			20								$2^{-1.00\pm0.00}$
Phenol‖	10min	16	4									$2^{0.80\pm0.41}$
	20min	9	11									$2^{0.45\pm0.51}$

§ Twenty strains were tested for each disinfectant. Figures in the table represent the number of strains showing the respective MBCs. The number of bacteria inoculated into each tube was $(3.88 \pm 1.66) \times 10^{8}$.

‖ Significant difference between values at 10 and 20 min. $p<0.05$.

Table 4. MBCs of six different disinfectants against *P. cepacia*§

Disinfectant	Concentration (%)	2^{1}	2^{0}	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}	MEAN ± S.D.
Benzethonium chloride	10min			3	4	2	4	3		2	2	$2^{-3.90\pm2.27}$
	20min			3	4	2	2	4		2	3	$2^{-4.15\pm2.46}$
Chlorhexidine gluconate	10min			7	6		3	3	1			$2^{-2.60\pm1.70}$
	20min			1	7	5	2	2	2	1		$2^{-3.35\pm1.66}$
Alkyldiaminoethyl-glycine hydrochloride	10min	2	2	3	4	3	2	1	3			$2^{-2.45\pm2.21}$
	20min	1	3	2	4	3	2	1	4			$2^{-2.75\pm2.24}$
Glutaraldehyde	10min				1	10	9					$2^{-3.40\pm0.60}$
	20min					7	13					$2^{-3.65\pm0.49}$
Povidone-iodine	10min			15	5							$2^{-1.25\pm0.44}$
	20min			14	6							$2^{-1.30\pm0.47}$
Phenol	10min	14	6									$2^{0.70\pm0.47}$
	20min	11	9									$2^{0.55\pm0.51}$

Twenty strains were tested for each disinfectant. Figures in the table represent the number of strains showing the respective MBCs. The number of bacteria inoculated into each tube was $(9.21 \pm 5.81) \times 10^{7}$.

Table 5. MBCs of six different disinfectants against *P. maltophilia*[§]

Disinfectant	Concentration (%)	2^{1}	2^{0}	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}	2^{-9}	MEAN ± S.D.
Benzethonium chloride	10min								1	5	12	2	$2^{-7.75\pm0.72}$
	20min								1	3	13	3	$2^{-7.90\pm0.72}$
Chlorhexidine gluconate	10min				1	3	12	2	1	1			$2^{-4.10\pm1.07}$
	20min					4	12	2		2			$2^{-4.20\pm1.11}$
Alkyldiaminoethyl-glycine hydrochloride	10min						2	3	14	1			$2^{-5.70\pm0.73}$
	20min							3	15	2			$2^{-5.95\pm0.51}$
Glutaraldehyde	10min			1	2	3	12	2					$2^{-3.60\pm0.99}$
	20min					3	14	3					$2^{-4.00\pm0.56}$
Povidone-iodine	10min			14	6								$2^{-1.30\pm0.47}$
	20min			14	6								$2^{-1.30\pm0.47}$
Phenol[‖]	10min	19	1										$2^{0.95\pm0.22}$
	20min	11	9										$2^{0.55\pm0.51}$

§ Twenty strains were tested for each disinfectant. Figures in the table represent the number of strains showing the respective MBCs. The number of bacteria inoculated into each tube was $(1.42 \pm 0.62) \times 10^{8}$.

‖ Significant difference between values at 10 and 20 min. $p<0.05$.

Table 6. MBCs of six different disinfectants against *A. xylosoxidans*[§]

Disinfectant	Concentration (%)	2^{1}	2^{0}	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	MEAN ± S.D.
Benzethonium chloride	10min		2	1	1	1	2	4	7	2	$2^{-4.50\pm2.19}$
	20min		2		2	1	1	3	8	3	$2^{-4.75\pm2.20}$
Chlorhexidine gluconate	10min			1	2	1	8	8	1		$2^{-4.00\pm1.17}$
	20min				3	1	4	11			$2^{-4.30\pm1.17}$
Alkyldiaminoethyl-glycine hydrochloride	10min	2			2	3	6	7			$2^{-3.50\pm1.82}$
	20min		2		2		8	7	1		$2^{-3.85\pm1.63}$
Glutaraldehyde[‖]	10min					5	13	2			$2^{-3.85\pm0.59}$
	20min						15	5			$2^{-4.25\pm0.44}$
Povidone-iodine	10min			11	9						$2^{-1.45\pm0.51}$
	20min			11	9						$2^{-1.45\pm0.51}$
Phenol	10min	12	8								$2^{0.60\pm0.50}$
	20min	9	11								$2^{0.45\pm0.51}$

§ Twenty strains were tested for each disinfectant. Figures in the table represent the number of strains showing the respective MBCs. The number of bacteria inoculated into each tube was $(2.65 \pm 1.31) \times 10^{7}$.

‖ Significant difference between values at 10 and 20 min. $p<0.05$.

Table 7. MBCs of six different disinfectants against *A. anitratus*§

Disinfectant	Concentration (%)	2^{1}	2^{0}	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}	2^{-9}	MEAN ± S.D.
Benzethonium chloride	10min								2	5	9	4	$2^{-7.75\pm0.91}$
	20min								1	3	8	8	$2^{-8.15\pm0.88}$
Chlorhexidine gluconate	10min					1	6	9	4				$2^{-4.80\pm0.83}$
	20min						7	5	8				$2^{-5.05\pm0.89}$
Alkyldiaminoethyl-glycine hydrochloride‖	10min						1	5	12	2			$2^{-5.75\pm0.72}$
	20min							1	8	10			$2^{-6.55\pm0.69}$
Glutaraldehyde	10min				2	3	15						$2^{-3.65\pm0.67}$
	20min					3	17						$2^{-3.85\pm0.37}$
Povidone-iodine	10min			18	2								$2^{-1.10\pm0.31}$
	20min			17	3								$2^{-1.15\pm0.37}$
Phenol	10min	14	6										$2^{0.70\pm0.47}$
	20min	9	11										$2^{0.45\pm0.51}$

§ Twenty strains were tested for each disinfectant. Figures in the table represent the number of strains showing the respective MBCs. The number of bacteria inoculated into each tube was $(1.65 \pm 1.28) \times 10^{8}$.

‖ Significant difference between values at 10 and 20 min. $p<0.05$.

The mean MBCs of all the disinfectants tested against each bacterial species, the MBCs effective for the most resisitant strain(s), the most resistant strain(s) among all the strains tested, and the concentrations recommended for hospital use by the manufacturers (The Japanese Pharmacopoeia, Tenth Revision, 1981) are summarized in Table 8.

Benzethonium chloride showed the highest efficacy expressed as mean MBC after both 10 and 20 min of exposure to the seven species of organisms. It was followed in order by chlorhexidine gluconate, alkyldiaminoethylglycine hydrochloride, glutaraldehyde, povidone-iodine, and phenol. Benzethonium chloride also showed the widest range of mean MBCs against the seven species of organisms, followed in order by alkyldiaminoethylglycine hydrochloride and chlorhexidine gluconate. These last-named three disinfectants showed a far wider range of mean MBCs compared with the other three disinfectants.

Benzethonium chloride, alkyldiaminoethylglycine hydrochloride and chlorhexidine gluconate has a wider range of MBCs against *P. cepacia* and *A. xylosoxidans* than against other species, showing even greater difference among strains. The most resistant strain(s) among those of *P. cepacia* and/or *A. xylosoxidans* against the above three disinfectants was (were) also the most resistant strain(s) among all the strains tested. In the case of *S. aureus* and *A. anitratus*, the bactericidal (and/or bacteriostatic against *S. aureus*) effect of each disinfectant did not show much difference among strains.

The minimum concentrations of benzethonium chloride, chlorhexidine gluconate, alkyldiaminoethylglycine hydrochloride and povidone-iodine recommended for hospital use by the manufacturers (0.1%, 0.02%, 0.1% and 0.75%, respectively) were not necessarily effective enough to kill all the strains or species tested (Table 9). At the minimum concentrations recommended for hospital use by the manufacturers, chlorhexidine gluconate showed the lowest efficacy followed in order by alkyldiaminoethylglycine hydrochloride, benzethonium chloride and povidone-iodine. Chlorhexidine gluconate was not bactericidal against many strains of all bacterial species tested, especially against strains of glucose-nonfermentative Gram-negative bacilli except for *P. aeruginosa*. Alkyldiaminoethylglycine hydrochloride was remarkably ineffective against *S. aureus* and *P. cepacia*. Benzethonium

Table 8. The mean MBCs of all the disinfectants tested against each bacterial species; the MBCs effective for the most resisitant strains; the most resistant strains among all the strains tested; and the concentrations recommended for hospital use by the manufacturers.

Disinfectant		Mean MBCs of all disinfectants tested (%)	MBCs effective for the most resistant strains (%)	The most resistant strains	Concentrations recommended for hospital use (%)
Benzethonium chloride	10min	$2^{-5.76\pm1.70}$	2^{0}	*A. xylosoxidans*	0.1~1
	20min	$2^{-6.06\pm1.65}$	2^{0}	*A. xylosoxidans*	($2^{-3.32}$~2^{0})
Chlorhexidine gluconate	10min	$2^{-4.62\pm1.14}$	2^{-1}	*P. cepacia*, *A. xylosoxidans*	0.02~1
	20min	$2^{-4.96\pm1.07}$	2^{-1}	*P. cepacia*	($2^{-5.64}$~2^{0})
Alkyldiaminoethyl-glycine hydrochloride	10min	$2^{-4.21\pm1.31}$	2^{1}	*P. cepacia*, *A. xylosoxidans*	0.1~0.5
	20min	$2^{-4.61\pm1.44}$	2^{1}	*P. cepacia*	($2^{-3.32}$~2^{-1})
Glutaraldehyde[||]	10min	$2^{-3.46\pm0.28}$	2^{-1}	*P. maltophilia*	2
	20min	$2^{-3.88\pm0.24}$	2^{2}	*P. aeruginosa*	(2^{1})
Povidone-iodine	10min	$2^{-1.25\pm0.26}$	2^{0}	*P. aeruginosa*, *S. aureus*	0.75~1
	20min	$2^{-1.29\pm0.23}$	2^{-1}	all species	($2^{-0.42}$~2^{0})
Phenol[†]	10min	$2^{0.81\pm0.15}$	2^{1}	all species	2~3
	20min	$2^{0.65\pm0.21}$	2^{1}	all species	(2^{1}~$2^{1.58}$)

|| Significant difference between values at 10 and 20 min. $P<0.05$.

Table 9. The number of strains to which the minimum concentrations of individual disinfectants recommended for hospital use by the manufacturers were not bactericidal.

Disinfectant	Species	*S. aureus*	*E. coli*	*P. aeruginosa*	*P. cepacia*	*P. maltophilia*	*A. xylosoxidans*	*A. anitratus*
Benzethonium chloride	10min	0	0	7	9	0	5	0
	20min	0	0	2	9	0	5	0
Chlorhexidine gluconate	10min	10	7	9	19	18	20	16
	20min	8	7	3	17	18	19	12
Alkyldiaminoethyl-glycine hydrochloride	10min	20	3	3	14	0	7	0
	20min	15	2	1	13	0	4	0
Glutaraldehyde	10min	0	0	0	0	0	0	0
	20min	0	0	0	0	0	0	0
Povidone-iodine	10min	2	0	1	0	0	0	0
	20min	0	0	0	0	0	0	0
Phenol	10min	0	0	0	0	0	0	0
	20min	0	0	0	0	0	0	0

Twenty stains were tested for each disinfectant.

chloride was not bactericidal to some strains of *P. cepacia*, *P. aeruginosa* and *A. xylosoxidans*. Povidone-iodine was not effective against 2 out of 20 strains of *S. aureus* and against 1 out of 20 strains of *P. aeruginosa* after only 10-min exposure. On the whole, among all the species tested, *P. cepacia* showed the highest resistance to all disinfectants tested at the minimum concentrations recommended for hospital use by the manufacturers, followed by *A. xylosoxidans* and S. *aureus*; *E. coli* showed the lowest resistance. Moreover, even when the maximum concentrations of each disinfectant recommended for hospital use were taken, alkyldiaminoethylglycine hydrochloride (0.5%) was not effective against 4 out of 20 strains of *P. cepacia* and against 2 out of 20 strains of *A. xylosoxidans* for both 10- and 20-min exposures.

Significant differences between MBCs after 10- and 20-min exposure were seen most often with the *P. aeruginosa* group among the bacterial species (with 4 out of 6 disinfectants tested, i.e., with benzethonium chloride, chlorhexidine gluconate, glutaraldehyde and phenol), and with glutaraldehyde among the disinfectants (against 4 out of 7 species tested, i.e., against *P. aeruginosa*, *A. xylosoixidans*, *E. coli* and *S. aureus*).

DISCUSSION

Previous investigators have reported mainly on the rapidity of bactericidal effect and/or bacteriostatic effect of various disinfectants.[1-5] In 1974 Kelsey et al. published an improved Kelsey-Sykes test, used to estimate the concentrations of disinfectants which may be recommended for hospital use.[22] In their test the most resistant organism was selected from the laboratory strains as a test organism and 3 percent Tween 80 in nutrient broth was used as the inactivator for all types of disinfectants. However, we have experienced that Tween 80 does not necessarily inactivate all types of disinfectants. It seems quite probable that there are often clinically isolated strains which are much more resistant to some disinfectants than the most resistant laboratory strains. Therefore, we did not use any inactivators nor any laboratory strains. Instead, we performed the control method simultaneously with the test to check the effect of the disinfectant carried over from the bacteria/disinfectant mixture into the recovery broth, and we used clinically isolated bacteria.

The bactericidal activity of each disinfectant varied from species to species and from strain to strain despite belonging to the same species. Although concentrations of disinfectants recommended for hospital use by the manufacturers differ somewhat, the lowest concentration should be the one which is effective enough to kill the most resistant strain. On the basis of our present results, the minimum concentrations for benzethonium chloride, chlorhexidine gluconate, alkyldiaminoethylglycine hydrochloride and povidone-iodine must be increased, and the minimum concentration of glutaraldehyde can be decreased (Table 8).

Benzethonium chloride exhibited the strongest bectericidal activity in terms of its mean MBC value against the seven species tested, but it showed the widest range of mean MBCs against the seven species, followed by alkyldiaminoethylglycine and chlorhexidine gluconate. These three disinfectants also showed wide ranges of MBCs against the individual strains of several species. For example, in the case of *P. cepacia*, the MBCs effective for the most resistant strains of both alkyldiaminoethylglycine hydrochloride and phenol were the same (2^1%), whereas the mean MBC of the former was significantly lower than that of the latter. Therefore, it cannot necessarily be concluded that alkyldiaminoethylglycine hydrochloride has stronger disinfectant bacericidal activity than phenol against *P. cepacia*. Phenol, povidone-iodine and glutaraldehyde proved to be excellent disinfectants with respect to less difference in their MBCs among species and among strains of each species.

There were several reports on contamination of disinfectant solution by Gram-negative bacilli resulting in outbreaks of nosocomial infections, especially contamination of chlorhexidine gluconate, benzalkonium chloride (a disinfectant belonging to the quaternary ammonium compound similar to benzethonium chloride) and povidone-iodine by *P. cepacia*.[12, 13, 15-18] In our present study MBCs against *P. cepacia* and *A. xylosoxidans* showed far more difference among strains with benzethonium chloride, alkyldiaminoethylglycine hydrochloride and chlorhexidine gluconate than with the remaining three disinfectants. Our results demonstrated that *P. cepacia* and *A. xylosoxidans* were apt to poduce resistant strains to these three disinfectants and thereby contaminate them. However, MBCs of povidone-

iodine against *P. cepacia* and *A. xylosoxidans* were restricted to a small range.

In the case of *P. aeruginosa*, there were significant differences between the mean MBCs after 10- and 20-min exposure with all the disinfectants tested, except alkyldiaminoethylglycine hydrochloride and povidone-iodine. Among the disinfectants tested, glutaraldehyde showed significant differences between its mean MBCs after 10- and 20-min exposure except against *P. cepacia*, *P. maltophilia* and *A. anitratus*. For the other combinations of the disinfectants and the bacterial species, there were no significant differences between the mean MBCs after 10- and 20-min exposure except the combinations of alkyldiaminoethylglycine hydrochloride and *A. anitratus*, and of phenol and *P. maltophilia*. These results suggest that, when disinfection of *P. aeruginosa* is the aim or when glutaraldehyde is used as a disinfectant, the time of exposure to the disinfectant is an important factor in its efficacy.

Berkelman et al. found that low concentrations of povidone-iodine (0.1 to 1%) were more rapidly bactericidal against *S. aureus* and *Mycobacterium chelonei* than 10% solution.[23] We exposed the test organisms to the disinfectant for 10- and 20-min and these exposure times were enough to show that low concentrations of povidone-iodine were less bactericidal than its higher concentrations. Berkelman's data shows that the MBC of povidone-iodine against *S. aureus* is 0.01% (ca. $2^{-6.64}$%) after 4-min exposure, far lower than our 10-min exposure result ($2^{-1.05}$%). This is probably due to the lower number of organisms in her inoculum/disinfectant mixture (10^5/ml) than that in ours (10^7/ml).

With *S. aureus*, there was inhibition of growth at the same, or lower, concentrations in some controls, as the last dilution showed no growth in the recovery broth for chlorhexidine gluconate and alkyldiaminoethylglycine hydrochloride. Therefore, the MBCs of these two disinfectants against *S. aureus* could not be determined. It is probable that the differences between the MBCs and MICs of these two disinfectants against *S. aureus* were very small.

In summary, selection of a disinfectant should be made bearing in mind the fact that the bactericidal activity of each disinfectant varies from species to species and from strain to strain of individual species. Therefore, the minimum concentration of the disinfectant for use must be one which can kill the most resistant strains among those to be disinfected. The minimum concentrations of each disinfectant which were able to kill all the strains tested (MBCs against the most resistant strains) for 10-min exposure in our study were 0.5% for glutaraldehyde and chlorhexidine gluconate, 1% for povidone-iodine and benzethonium chloride, 2% for alkyldiaminoethylglycine hydrochloride and phenol, and the MBCs effective for all the tested strains for 20-min exposure were 0.25% for glutaraldehyde, 0.5% for chlorhexidine gluconate and povidone-iodine, 1% for benzethonium chloride, and 2% for alkyldiaminoethylglycine hydrochloride and phenol.

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