STABILITY AND BACTERICIDAL ACTIVITY OF CHLORINE SOLUTIONS

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ABSTRACT

OBJECTIVES: To determine the stability of sodium hypochlorite (diluted household bleach) when stored for 30 days in various types of containers and to determine the efficacy of low concentrations of free available chlorine to inactivate test bacteria.

DESIGN: Laboratory-based study. Solutions of standard household bleach were prepared using tap water or sterile distilled water at dilutions of 1:100, 1:50, and 1:5. Chlorine concentrations were measured, and then the solutions were placed into five polyethylene containers and left at room temperature (20°C) under various conditions (translucent containers with light exposure and with or without air; brown opaque container without light or air exposure). Samples for chlorine and pH determinations were taken at time 0 and on days 7, 14, 21, 30, and 40. Bactericidal activity of chlorine solutions was assessed using the Association of Official Analytical Chemists Use-Dilution Method. Test bacteria included *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella choleraesuis.*

RESULTS: Chlorine concentrations at 30 days varied from the 40% to 50% range for 1:50 or 1:100 dilutions stored in containers other than closed brown containers to 83% to 85% for the 1:5 dilution stored in closed but nonopaque containers to 97% to 100% for 1:50 or 1:5 solutions stored in closed brown containers. The lowest concentration of sodium hypochlorite solution that reliably inactivated all the test organisms was 100 ppm.

CONCLUSIONS: These data suggest that chlorine solutions do not need to be prepared fresh daily, as is recommended currently, and the lowest concentration of chlorine that reliably inactivates *S aureus, S choleraesuis,* and *P aeruginosa* is 100 ppm (*Infect Control Hosp Epidemiol* 1998;19:323-327).

Hypochlorite (chlorine) solutions are used widely in hospitals, particularly for cleaning spills of blood or bloody body fluids, because they inactivate the human immunodeficiency virus (HIV) and hepatitis B virus.[1,2] The Centers for Disease Control and Prevention (CDC) has recommended that a freshly prepared solution of sodium hypochlorite (household bleach) be used at chlorine concentrations from 500 to 5,000 ppm (1:100 to 1:10 dilution of household bleach) to clean blood spills, depending on the amount of organic matter present on the surface to be cleaned or disinfected.[3] In addition, hypochlorite solutions have been recommended to disinfect cardiopulmonary resuscitation manikins, countertops in the dietary department, hydrotherapy tanks, laundry, tonometers, diaper surface areas in day-care centers, and dental impressions.[1,2,4]

Hypochlorites are the most widely used chlorine disinfectants in the United States. They have a broad spectrum of antimicrobial activity and are both inexpensive and fast acting.[1] However, their use in hospitals is limited by their corrosiveness, inactivation by organic matter, and instability.[1,5-8] Decomposition of hypochlorite solutions leads to a loss of available chlorine and hence to a loss of antimicrobial activity. Decomposition is affected by many factors, including temperature, chlorine concentration, light, presence of catalysts, and, most importantly, pH.[5,8] The dissociation of hypochlorous acid to the less microbicidal form, hypochlorite ion (OCl^-), is dependent on pH.

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The disinfecting efficacy of chlorine decreases with an increase in pH, in parallel with the conversion of undissociated hypochlorous acid to OCl^-.

The purpose of this study was to determine the stability of hypochlorite solutions and the ability of freshly prepared and 30-day-old solutions to inactivate bacteria.

METHODS

We used the Association of Official Analytical Chemists (AOAC) Use-Dilution Method (UDM) to assess the effectiveness of freshly prepared and 30-day-old chlorine solutions against selected test bacteria.[9] The UDM, until recently, was the standard test required by the Environmental Protection Agency (EPA) to assess bactericidal activity of germicides. In brief, stainless-steel penicylinders are inoculated by soaking for 15 minutes in a 48-hour broth culture of test bacteria. The penicylinders are removed with a hooked inoculating needle and allowed to dry for 40 minutes at 37°C. After drying, the inoculated penicylinders are placed individually into the disinfectant solution and exposed for 10 minutes. The penicylinders are removed carefully and placed into tubes containing 10 mL of neutralizing broth. Each penicylinder was contaminated with 10^6-10^7 bacteria.[10] The tubes are incubated for 48 hours and then examined for turbidity. The number of penicylinders used at each chlorine dilution varied; larger numbers were used when testing chlorine concentrations more commonly used in healthcare. In general, replicates were processed in multiples of 10. Occasionally, technical problems led to the exclusion of a sample from the analysis. Using current EPA pass-fail specifications, greater than one positive penicylinder out of 60 replicates indicates that the dilution tested is not adequate to obtain the required bactericidal activity, and the disinfectant fails.

The three recommended AOAC Use-Dilution Test bacteria were used: *Pseudomonas aeruginosa* #15442; *Staphylococcus aureus* #6538; and *Salmonella choleraesuis* #10708 (American Type Culture Collection, Rockville, MD). The media used in the testing were those specified in the AOAC UDM. A 5.25% sodium hypochlorite solution (household bleach) was obtained from a commercial source. Water used to prepare the hypochlorite solutions was either glass distilled water (Travenol, Deerfield, IL) or tap water, as indicated. The hardness of the tap water was 30 ppm as calcium carbonate.

Sodium hypochlorite solutions were stored in 500-mL polyethylene containers (Figure 1) and kept at room temperature (22±1°C). Five types of containers were used, including a translucent open bottle, a translucent closed bottle with a screw cap, a brown opaque bottle with a screw cap, a translucent wash bottle, and a translucent spray bottle. All solutions were stored on a laboratory bench in a room with closed windows. The solutions (except the brown bottle) were exposed primarily to indirect sunlight but occasionally to direct sunlight. At the indicated intervals, samples were assayed for free available chlorine (FAC).

Determination of FAC was performed using the standard N, N-diethyl-p-phenylenediamine ferrous ammonium sulfate method (Figures 2, 3, and 4) or amperometric titration (Table 2) with 0.00564 mol/L phenylarsine oxide solution.[11] All glassware was rendered chlorine-demand free. The pH was measured with a digital pH meter (Model 701, Orion Research, Beverly, MA) that was standardized before each use. A single titration was performed to determine the chlorine concentrations reported in Tables 1 and 2.

RESULTS

Stability of Hypochlorite Solutions

For bleach diluted 1:100 with tap water, the concentration of available chlorine was reduced to 40% to 42% of the original concentration when stored in translucent spray or wash bottles for 30 days (Figure 2; Table 1). Similar results were obtained using a 1:50 dilution with tapwater. That is, the concentration of available chlorine was reduced to 47% of the original concentration when stored in spray or wash bottles for 30 days (Figure 3; Table 1).

When diluted 1:5 with tapwater, the concentration of available chlorine was reduced to approximately 85% of the original concentration when stored in closed spray or wash bottles for 30 days, and to 46% when stored in an open bottle for 30 days. There was no deterioration of the hypochlorite solution after 30 days when stored in the closed brown bottle (Figure 4; Table 1).

Bactericidal Activity of Hypochlorite Solutions

The lowest concentration of sodium hypochlorite solution that reliably inactivated the three AOAC test organisms (*P aeruginosa, S aureus,* and *S choleraesuis)* using the AOAC UDM was 100 ppm (Table 2). Solutions prepared at a concentration of 500 ppm (1:100), which contained, after 30 days, 255 ppm chlorine in distilled water and 225 ppm in tap water, killed 10^7 *P aeruginosa* using the AOAC UDM (Table 2).

DISCUSSION

Hypochlorite solutions are used widely in hospitals, because the CDC recommends their use on blood spills. Chlorine solutions have broad antimicro-

TABLE 1
EFFECT OF CONTAINER TYPE AND DILUTION OF SODIUM HYPOCHLORITE ON FREE AVAILABLE CHLORINE LEVELS

Dilution	Bottle Type	pH	Free Available Chlorine*		
			Initial	30 Days	% Remaining at 30 Days
1:100	Spray	9.2-8.2	475	190	40
	Wash	9.2-8.1	475	200	42
1:50	Open	9.5-8.4	1,050	560	53
	Closed	9.5-9.3	1,050	490	47
	Spray	9.5-8.7	1,050	490	47
	Wash	9.5-8.6	1,050	495	47
	Closed brown	9.5-9.4	1,050	1,020	97
1:5	Open	10.6-8.8	10,250	4,750	46
	Closed	10.6-10.5	10,250	8,750	85
	Spray	10.6-10.1	10,250	8,750	85
	Wash	10.6-10.1	10,250	8,500	83
	Closed brown	10.6-10.5	10,250	10,250	100

* Measured as per methods section.

Figure 1. Solutions (350 mL) were placed into five polyethylene containers and left at room temperature. Bottles from left to right are translucent, screw-capped, 500 mL, open (air and light exposure); translucent, screw-capped, 500 mL, closed (light exposure only); brown opaque, screw-capped, 500 mL, closed (no light or air exposure); translucent wash bottle, 500 mL; and translucent spray bottle, 500 mL.

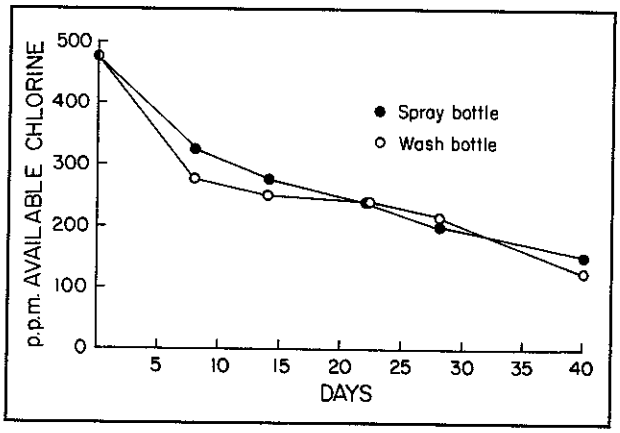

Figure 2. Effect of container type on decomposition of 1:100 dilution of 5.25% sodium hypochlorite.

bial activity that includes the ability to inactivate HIV and hepatitis B. CDC guidelines recommend that chlorine solutions be made fresh daily.[2] However, in many settings, diluted chlorine solutions are made periodically and stored for use in a variety of containers. Our experiments provide data on changes in free chlorine concentrations over time, and how these changes are altered by the type of storage bottle.

Many factors affect the stability of FAC in solution. These include chlorine concentration; presence and concentration of catalysts (eg, heavy-metal ions); pH of the solution; temperature of the solution; presence of organic material; and ultraviolet irradiation.[5,8] The literature states that most stable FAC solutions have the following characteristics: low chlorine concentrations; absence of copper, nickel, cobalt, or other catalysts of decomposition; high alkalinity; low temperature; absence of organic material; and storage in dark and closed containers (ie, shielded from ultraviolet light). The hardness of the water (ie, the presence of Mg^{++} and CA^{++} ions) does not have a significant effect on the antibacterial action of hypochlorite solutions. Our study revealed that, over the range of chlorine concentrations tested, the most stable concentration was the highest concentration tested (ie, 1:5 dilution). It also appears that the stability results noted in Table 1 were affected by the pH. Solutions with the lowest pH exhibited the fastest decay of chlorine. The pH decreases demonstrated in our study could

TABLE 2
BACTERICIDAL ACTIVITY OF VARIOUS CONCENTRATIONS OF HYPOCHLORITE SOLUTIONS AGAINST *PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS,* AND *SALMONELLA CHOLERAESUIS* USING THE USE-DILUTION METHOD

	Distilled Water		Tap Water	
	Free Chlorine Approximate (Exact) PPM	Positive Carriers/Replicates	Free Chlorine Approximate (Exact) PPM	Positive Carriers/Replicates
Pseudomonas aeruginosa				
Freshly prepared	1 (.92)	10/10	1 (2.6)	10/10
	10 (13)	8/10	10 (10.5)	9/10
	50 (46)	1/10	50 (51.5)	1/10
	(46) 0/19			
	100 (96)	0/20	ND	
	(93) 0/60			
	200 (186)	0/20	ND	
	450 (440)	1/60		
	(470) 0/20	ND		
30-day-old	(255) 0/20	(225)	1/20	
Staphylococcus aureus				
	10 (9.4)	19/19	ND	
	50 (48)	5/19		
	100 (97)	1/20		
	(93) 2/60			
	450 (465)	0/59		
	(440) 0/60			
Salmonella choleraesuis				
	10 (9)	1/20	ND	
	50 (49)	1/18		
	100 (96)	0/20		
	(89) 0/58			
	450 (440)	0/60		

Abbreviation: ND, no data; PPM, parts per million free available chlorine.
Association of Official Analytical Chemists Use Dilution Method pass criteria are ≤1 positive penicylinder per 60 replicates. A previous study[10] demonstrated that the microbial load on penicylinders is as follows: *P aeruginosa,* 1.4×10^7; *S aureus,* 6×10^6; and *S choleraesuis,* 8×10^5.

occur by three mechanisms: dilution with water (the greater the dilution, the lower the pH); adsorption of carbon dioxide through exposure to air (especially for the 1:5 dilution); and light-catalyzed decomposition of hypochlorite. Our data for the 1:5 dilution shows that, by itself, light is not too detrimental; but, as shown with the 1:50 dilution, a decreased pH results from light-catalyzed decomposition, which greatly accelerates the loss of hypochlorite.

The conditions of chlorine solution use in hospitals favor stability of chlorine availability, including use at room temperature, use of relatively diluted solutions, alkaline pH range, closed containers, absence of catalysts known to promote decomposition, and often storage in opaque containers. Our data further confirm that closed, brown, opaque bottles were superior to translucent bottles. However, all of the bottles commonly used in healthcare settings allowed substantial retention of chlorine activity at 30 days. For example, the 30-day-old solutions from the open bottles (255 ppm available chlorine in distilled water and 225 ppm available chlorine in tap water) killed 10^7 *P aeruginosa.*

Based on these data and those of Hoffman et al,[8] daily preparation of bleach solutions is not necessary to ensure biocidal activity. The FAC levels of solutions in translucent containers were reduced at worst to 40% of the original concentration at 30 days. Although these studies should be corroborated with tap water from other geographic locations before being accepted universally, it appears that the user would have the required amount of available chlorine at day 30 if they prepared a solution twice (or 2×) as

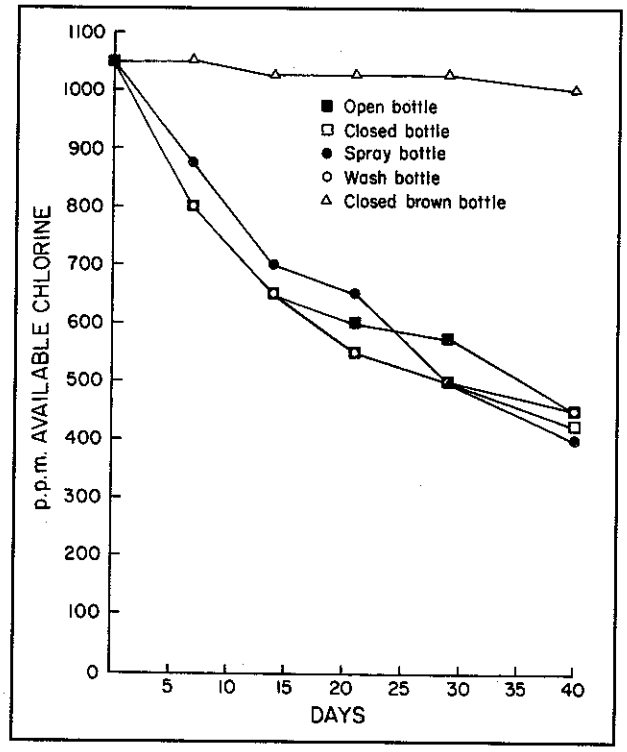

Figure 3. Effect of container type on decomposition of 1:50 dilution of 5.25% sodium hypochlorite.

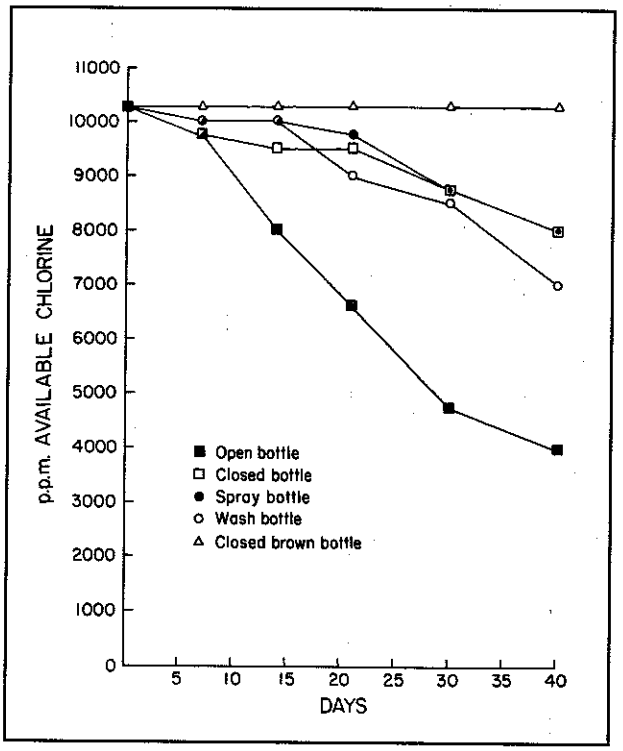

Figure 4. Effect of container type on decomposition of 1:5 dilution of 5.25% sodium hypochlorite.

strong as desired at time 0. For example, if you wished to have a solution containing 500 ppm of available chlorine at day 30, prepare a solution containing 1,000 ppm of chlorine at time 0. Chlorine solutions stored in closed, opaque containers are stable for a minimum of 30 days.

The AOAC test was used to measure the bactericidal activity of chlorine solutions. The lowest concentration of sodium hypochlorite solution that reliably inactivated all three test organisms (ie, *P aeruginosa, S aureus,* and *S choleraesuis)* was 100 ppm. All penicylinders contaminated with either *P aeruginosa* or *S choleraesuis* were sterilized by 100 ppm chlorine. Despite being contaminated with a very high number of *S aureus,* 6×10^6, only 3.75% of penicylinders revealed growth in a system theoretically able to identify a single viable organism. Excellent activity against *P aeruginosa, S aureus,* and *S choleraesuis* was demonstrated at 50 ppm and against *S choleraesuis* at 10 ppm. These concentrations are 50 to 100 times lower than that obtained by the standard 1:10 dilution of household bleach. These higher concentrations are reasonable, because organic material that may be present on environmental surfaces consumes available chlorine and reduces its capacity for bactericidal activity.[6] The effect of an organic load such as a blood spill on the efficacy of chlorine solutions should be assessed further.

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