

Research article

## The efficacy of chemical agents in cleaning and disinfection programs

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Published: 24 September 2001

Received: 3 August 2001

*BMC Infectious Diseases* 2001, 1:16

Accepted: 24 September 2001

This article is available from: <http://www.biomedcentral.com/1471-2334/1/16>

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### Abstract

**Background:** Due to the growing number of outbreaks of infection in hospital nurseries, it becomes essential to set up a sanitation program that indicates that the appropriate chemical agent was chosen for application in the most effective way.

**Method:** For the purpose of evaluating the efficacy of a chemical agent, the minimum inhibitory concentration (MIC) was reached by the classic method of successive broth dilutions. The reference bacteria utilized were *Bacillus subtilis* var. *globigii* ATCC 9372, *Bacillus stearothermophilus* ATCC 7953, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923. The strains of *Enterobacter cloacae* IAL 1976 (Adolfo Lutz Institute), *Serratia marcescens* IAL 1478 and *Acinetobacter calcoaceticus* IAL 124 (ATCC 19606), were isolated from material collected from babies involved in outbreaks of infection in hospital nurseries.

**Results:** The MIC intervals, which reduced bacteria populations over 08 log<sub>10</sub>, were: 59 to 156 mg/L of quaternarium ammonium compounds (QACs); 63 to 10000 mg/L of chlorhexidine digluconate; 1375 to 3250 mg/L of glutaraldehyde; 39 to 246 mg/L of formaldehyde; 43750 to 87500 mg/L of isopropanol or ethanol; 1250 to 6250 mg/L of iodine in polyvinyl-pyrrolidone complexes, 150 to 4491 mg/L of chlorine-releasing-agents (CRAs); 469 to 2500 mg/L of hydrogen peroxide; and, 2310 to 18500 mg/L of peracetic acid.

**Conclusions:** Chlorhexidine showed non inhibitory activity over germinating spores. *A. calcoaceticus*, was observed to show resistance to the majority of the agents tested, followed by *E. cloacae* and *S. marcescens*.

### Background

The Ministry of Health in Brazil is concerned about hospital infections, which have involved an average of 13 of every 100 patients admitted into hospitals of the Brazil-

ian National Health System (SUS), the maximum tolerable being 5.7% according to the World Health Organization (WHO) [1]. Had good hygienic practices and the rational use of antibiotics been employed, it is es-

estimated that 1/3 of the cases could have been avoided. Furthermore, the government lacks sufficient information on the incidence of hospital infections in different regions of the country, so in 1998, the Ministry of Health made it mandatory to notify such cases. Unfortunately, less than 20% of Brazilian hospitals have adequate hospital infection control structures and preventive programs [2].

In 1997, the Pan American Health Organization (PAHO) [3] stated that 17 million people worldwide were fatal victims of hospital infections in 1995, as a consequence of the abandonment of sanitary vigilance programs and a reduction of investments in basic sanitation projects. PAHO [4] alerted that more than 250,000 children, under 5 years of age die annually in the Americas, from illnesses that can be easily prevented or treated.

In October 1996, according to feces and urine samples collected from new born babies, the genera *Acinetobacter calcoaceticus*, which can be fatal in 48 h to infected infants, was confirmed as the responsible agent for the death of infants in maternity hospitals in Boa Vista (Rondonia). Two babies hospitalized in November 1997, in a São Paulo Neonatal Intensive Care Unit, were infected by *Serratia marcescens*, which was the cause of 15 deaths of new born babies since August 1997. A further eleven babies, although infected during this period, reacted following antibiotic treatment. In November 1997, *Enterobacter cloacae* was confirmed to have infected 10 babies in a São Paulo Maternity. This Hospital has an average of 400 births monthly, ten percent of which could be infected. The bacterium was also involved in six deaths of infected babies in less than a week in October 1999, in a Rio de Janeiro Maternity. In a short period of time (October 1999 to January 2000), at least 24 deaths of babies from hospital infections were notified in Brazil. The origins of the infection by *A. calcoaceticus*, *E. cloacae* and *S. marcescens* remain uncertain, regardless of the fact that cross-contamination maintains the spread of the microorganism [3,5]. These data are frightening since they reflect a disheartening tendency for old microorganisms to reappear.

PAHO, WHO, national and international agencies and non-governmental organizations (NGOs) hope to prevent 100,000 deaths in children under 5 years of age in the Americas by 2002 by improving the training of health professionals, the quality of the health system required for effective administration of childhood illnesses and family and community practices [4].

Efforts to diminish the risk of transmission of infections include programs in which these infections have a crucial role. The surfaces of hospital medical devices, the skin of

the personnel, equipment, furniture and areas should be disinfected by an appropriate disinfecting agent and one which is easily acquired and handled, is harmless and easily disposed of should be chosen. But it is difficult to select an appropriate disinfecting agent because there is a large variety on the market, therefore it is imperative to identify the principal microorganisms encountered in health care centers before making the selection.

For the purpose of evaluating the range of activity of commercial sanitation products for hospital use against the bacteria involved in the deaths of new born babies in Brazil, the minimum inhibitory concentration (MIC) was determined by the classical method of successive broth dilutions.

## Methods

### Cultures of microorganisms

The bacterial strains, obtained from the collection of lyophilized cultures by the Adolfo Lutz Institute (IAL, SP, Brazil), were *Enterobacter cloacae* IAL 1976; *Serratia marcescens* IAL 1478; *Acinetobacter calcoaceticus* ATCC 19606, IAL 124. The reference bacteria used were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* var. *globigii* ATCC 9372, and *Bacillus stearothermophilus* ATCC 7953. Working cultures were kept on Tryptic soy agar (TSA, Difco, Detroit, Michigan, USA) at 4°C with weekly transfers. The 24 h cultures, developed on TSA, at 22°C for *S. marcescens*, and at 35–37°C for *E. cloacae*, *A. calcoaceticus*, *E. coli*; *S. aureus*, were harvested in Tryptic soy broth (TSB, Difco), centrifuged (1000 g/ 15 min/ 4°C) and suspended in saline. Bacteria viability was estimated through pour plate on TSA, confirming populations over 10<sup>7</sup> CFU/mL. Spore cultures, developed for 6-days on a sporulation medium (g.L<sup>-1</sup>: D-glucose (Sigma, St. Louis, Missouri, USA), 2.5; L-glutamic acid (Sigma), 0.4; yeast extract (Difco), 4.0; peptone (Difco), 5.0; sodium chloride, 0.01; manganese sulfate, 0.01; bacteriologic (Difco) agar, 20.0), at 37°C for *B. subtilis*, and at 62°C for *B. stearothermophilus*, were harvested, centrifuged (1935 g for 30 min, four times), and kept suspended in cooling 0.02 M calcium acetate solution (pH = 9.7) at 4°C [6]. The viability of heat-shocked (80°C/10 min for *B. subtilis* and 100°C/ 20 min for *B. stearothermophilus*) spores was obtained through pour plate on TSA, confirming populations over 10<sup>6</sup> spores/mL.

### Chemical agents

Chlorhexidine digluconate (biguanide, 1.6-dichlorophenyldiguanido hexane; 40% w/v; Zeneca Farmacêutica, SP, Br); hydrogen peroxide (40% w/v, Laborasa Farmacêutica, SP, Br), kept at 4°C; isopropyl alcohol (70% v/v, Johnson and Johnson -J&J -, SP, Br) and sodium dichloro-isocyanurate (NaDCC, sodium salt 50% w/w in tab-

lets, J&J) were tested. The following solutions were from Aster Produtos Medicos (SP, Br): ethanol (70%, v/v); ethanol (70% v/v) plus glycerin (2% w/v); glutaraldehyde (1.5 pentanedial; 2.0% w/v alkalized with sodium bicarbonate, pH = 8.3), formaldehyde (monoaldehyde; 37% w/v); sodium hypochlorite (10% w/v); peracetic acid (37% w/v) kept at 4°C; quaternarium ammonium compounds – QACs (10%w/v, benzalkonium chloride, monoquaternary mixture of alkyldimethylbenzylammonium chlorides); aqueous polyvinylpyrrolidone-iodine (10% w/v, PVP-I<sub>2</sub>, topic and soap) solution, with and without sodium lauryl ether sulfate (25 % w/v); alcoholic polyvinylpyrrolidone-iodine (10% w/v, PVP-I<sub>2</sub>, alcoholic) in ethanol 70% (v/v). Ethanol at 70% v/v, added with iodine 1% w/v and 10% w/v; and sodium hypochlorite at pH 7.0 in Sorensen phosphate-buffered solution were prepared at the laboratory. The concentration of total available chlorine, iodine and hydrogen peroxide was determined by the iodometric method [7]. The diluted so-

lutions, prepared with chlorine demand-free glassware, were filtered through a 0.22- $\mu$ m-pore-size membrane (Millipore, Bedford, MA, USA) for daily use. The chemical agents have their usage for hospital disinfection purposes approved by the Brazilian Ministry of Health [8].

#### Minimal inhibitory concentration

The minimal inhibitory concentration (MIC) was determined by using the two-fold broth dilution method [9]. Starting from a chemical agent solution, serial dilutions were prepared in TSB inoculated with the test bacterial populations  $\geq 10^6$  CFU/mL. The MIC was identified as the lowest concentration of the chemical agent, which resulted in confirmed inhibition of the growth of the tested microorganism, after 24 h of optimal incubation conditions. The chemical agent solutions, started concentrations and pH values are presented in Table 1. The MICs were expressed in percentage and in mg/L and are shown in Table 2.

**Table 1: Started solutions of chemical agents, their concentrations and pH values, which were used for the MIC tests.**

Chemical Agent	Solution Concentrations (%)	Solution pH values
QACs – Quaternarium Ammonium Compounds	0.5	6.3–6.9
Chlorhexidine digluconate	4.0, 0.1, 1.0	6.18–6.95
Glutaraldehyde	2.0, 1.5	7.14
Formaldehyde	0.5, 2.3	7.79
Alcohol: Ethanol (EtOH) or Isopropanol	70.0	5.50 (EtOH)
	70.0	6.59 (Isopropanol)
Ethanol (EtOH) plus glycerin	70.0 (EtOH) + 2.0 (glycerin)	5.94
Ethanol (EtOH) plus iodine	70.0 (EtOH) + 1.0 (I <sub>2</sub> )	5.50
	70.0 (EtOH) + 10.0 (I <sub>2</sub> )	5.50
Pvp-I <sub>2</sub> – topic: Aqueous polyvinylpyrrolidone-iodine	10.0 (I <sub>2</sub> )	5.32–5.95
Pvp-I <sub>2</sub> – soap – Aqueous Pvp-I <sub>2</sub> into lauryl ether sulfate (25% w/v)	10.0 (I <sub>2</sub> )	5.72
Pvp-I <sub>2</sub> – Alcoholic Pvp-I <sub>2</sub> into ethanol (70% v/v)	10.0 (I <sub>2</sub> )	2.04–2.90
CRAs – Chlorine releasing agents – Sodium hypochlorite (NaOCl) into phosphate buffer at pH = 7.0	1.0 total free chlorine	> 9.0
	0.1 total free chlorine	7.0–7.6
NaDCC – Sodium dichloro-isocyanurate	1.0 total free chlorine	6.70
H <sub>2</sub> O <sub>2</sub> – Hydrogen Peroxide	4.0	3.3
Peracetic Acid (PAA) at 3.7%. pH = 3.03.	3.7	3.03

## Results and Discussion

The chemical agents studied in commercial sanitation products are extensively used in health care settings for topical and hard-surface applications.

#### Quaternarium ammonium compounds – QACs

QACs are not sporocidal, their activity exhibiting inhibition of the outgrowth [10] of germinating spores. The MICs of 0.0117–0.0156 % (117–156 mg/L) for *B. subtilis* and *B. stearothermophilus* were twice those exhibited by vegetative cells of *E. cloacae*, *E. coli*, *S. aureus* and *S.*

*marcescens*, with a MIC ranging from 0.0059–0.0078% (59–78 mg/L), four times greater than the MIC (9.77 mg/L) observed for *A. calcoaceticus*.

#### Comments

In health care settings, the QACs, considered low-level disinfectants, are used in a minimum concentration of 0.2% (2000 mg/L), without formaldehyde in their formulation [8]. The QACS are mostly applied as antiseptic and on hard surfaces due to being non aggressive and releasing residual antimicrobial activity, respectively, re-

**Table 2: The minimum inhibitory concentrations (MIC) for the chemical agent solutions to reduce bacteria populations over 6-log<sub>10</sub>.**

Bacteria	<i>Bacillus stearothermophilus</i>		<i>Bacillus subtilis</i>		<i>Acinetobacter calcoaceticus</i>		<i>Enterobacter cloacae</i>		<i>Escherichia coli</i>		<i>Serratia marcescens</i>		<i>Staphylococcus aureus</i>	
	MIC	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L
Agent QACs	156	0.0156	117	0.0117	9.77	0.001	78	0.0078	59	0.0059	59	0.0059	59	0.0059
Chlorhexidine	*	*	10000	1.0	63	0.0063	71	0.0071	71	0.0071	141	0.0141	71	0.0071
Glutaraldehyde	1875	0.1875	3250	0.325	3250	0.325	3250	0.325	3250	0.325	1375	0.1375	1875	0.1875
Formaldehyde	246	0.0246	235	0.0235	39	0.0039	117	0.0117	156	0.0156	58.5	0.00585	156	0.0156
Alcohol	87500	8.75	87500	8.75	43750	4.375	87500	8.75	65650	6.565	43750	4.375	87500	8.75
EtOH (+ glycerin)	*	*	*	*	87500	8.75	87500	8.75	87500	8.75	87500	8.75	87500	8.75
EtOH (+I <sub>2</sub> 1%)	87500	8.75	87500	8.75	43750	4.375	87500	8.75	43750	4.375	43750	4.375	43750	4.375
I <sub>2</sub> 1% in EtOH	1250	0.125	1250	0.125	1250	0.125	1250	0.125	625	0.0625	625	0.0625	625	0.0625
EtOH (+ I <sub>2</sub> 10%)	-	-	43750	4.375	-	-	43750	4.375	-	-	-	-	21870	2.187
I <sub>2</sub> 10% in EtOH	-	-	6250	0.625	-	-	6250	0.625	-	-	-	-	3125	0.3125
Pvp-I <sub>2</sub> -topic	12500	1.25	50000	5.0	12500	1.25	6250	0.625	12500	1.25	6250	0.625	6250	0.625
Pvp-I <sub>2</sub> – soap	6250	0.625	50000	5.0	6250	0.625	6250	0.625	6250	0.625	6250	0.625	6250	0.625
Pvp-I <sub>2</sub> – alcoholic	12500	1.25	25000	2.5	12500	1.25	3125	0.3125	1560	0.156	3125	0.3125	3125	0.3125
CRA 1% pH > 9	4491	0.4491	4491	0.4491	867	0.0867	420	0.0420	1129	0.1129	474	0.0474	945	0.0945
CRA 0.1% pH 7	621	0.0621	621	0.0621	150	0.015	150	0.015	150	0.015	150	0.015	150	0.015
NaDCC	5990	0.599	5990	0.599	1123	0.1123	374	0.0374	1123	0.1123	187	0.0187	749	0.0749
H <sub>2</sub> O <sub>2</sub>	1875	0.1875	1875	0.1875	469	0.0469	1250	0.125	2505	0.2505	625	0.0625	938	0.0938
Peracetic acid	4620	0.4620	18500	1.85	9250	0.925	9250	0.925	2310	0.231	9250	0.925	4620	0.462

Legend: QACs – Quaternarium Ammonium Compounds; H<sub>2</sub>O<sub>2</sub> – Hydrogen peroxide Alcohol – Ethanol (EtOH) or Isopropanol; EtOH + I<sub>2</sub> (iodine); Pvp-I<sub>2</sub> – polyvinylpyrrolidone-iodine CRA – Chlorine releasing agents (CRA) – Sodium hypochlorite (NaOCl); NaDCC – Sodium dichloro-isocyanurate (\* no efficacy)

placing phenol compounds. In hospital nurseries, the QACs should be used in the disinfection of beds, matrix and general hard surfaces in order to minimize cross-contamination and the risk of exceeding super population, which occurs frequently in the SUS Hospitals.

**Chlorhexidine digluconate**

In Brazilian Hospitals, the concentration of 4.0% chlorhexidine solution is used for hand washing by the medical staff. In our study, chlorhexidine showed no efficacy over *B. stearothermophilus*, and a MIC of 1.0% for *B. subtilis*. The MIC interval of 0.0063–0.0071% (63–71 mg/L) was observed for *E. cloacae*, *E. coli* and *S. aureus*,

and for *S. marcescens*, the MIC was 0.0125–0.0156% (125–156 mg/L), therefore double that of the former.

**Comments**

Solution of chlorhexidine at 4%, containing 4% of alcohol (to prevent cross contamination with proteus and pseudomonas) [8], is widely applied for degermination of the hands of personnel and topic for pre-surgical areas. An alcoholic solution of chlorhexidine at 0.5% is indicated as a topical antiseptic, to replace PVP-I<sub>2</sub> allergic cases and for the bath of the newborn, after pre-degermination by chlorhexidine at 4%.

Chlorhexidine is also used for cleaning contact lenses (0.005–0.006%). To reduce populations greater than 5  $\log_{10}$  of *S. marcescens*, a MIC of 0.003% was reported to be equivalent to a 10 min exposure at a solution of 0.05% [11]. After 24 h contact time, the ability of *S. marcescens* to grow in chlorhexidine solutions at an interval of 0.001% to 0.006% was demonstrated [12].

#### **Glutaraldehyde (1.5-pentanedial)**

Glutaraldehyde, which exhibits high bactericidal, sporocidal, fungicidal, virucidal effectiveness, is widely used for high level disinfection of critical and semi-critical medical facilities, at a 2.0% concentration of an activated alkaline (pH = 8.3) solution, for 6–10 h exposure [8]. To reduce populations over 08  $\log_{10}$ , the MICs were between 0.275% – 0.375% (2750 – 3750 mg/L) for *B. subtilis*, *A. calcoaceticus*, *E. cloacae*, and *E. coli*; an interval MIC of 0.1375–0.1875% (1375 – 1875 mg/L) for *S. marcescens*, *S. aureus* and *B. stearothermophilus*.

#### **Comments**

Despite its antimicrobial effectiveness and beneficial annual cost, the side-effects of exposure to glutaraldehyde demand a safe work environment and a training program to assure worker safety standards [13], as well as its neutralization before pouring solutions down the drain [14]. Once all the residuals have been removed from the items by proper rinsing, glutaraldehyde will no longer represent a danger to the user.

#### **Formaldehyde (monoaldehyde)**

Formaldehyde is also selected for disinfection and airborne sanitization due to its antimicrobial (sporocidal, virucidal, fungicidal) activities. We observed that, for overnight decontamination of items, the use of a 0.5 to 1.0% formaldehyde aqueous solution provided a population reduction of 6–9  $\log_{10}$ , by an average MIC of 58.5 mg/L for *S. marcescens*; of 117 mg/L for *E. cloacae*; of 156 mg/L for *S. aureus* and *E. coli*; of 235 mg/L for *B. subtilis*, and of 246 mg/L for *B. stearothermophilus*.

#### **Comments**

The use of formaldehyde, in Brazil [8], is permitted for high level disinfection of critical and non critical items articles, such as dialysis machines and reused dialyzers; drains and tubes in general, catheters; laparoscopes; arthroscopes and ventiloscopes, acrylic grafts. These articles should be immersed for 18 h, in an 8% formaldehyde alcoholic solution; followed by abundant rinsing in water or sterile physiologic solution or alcohol, preventing recontamination. In the preparation of parenteral solutions, water for injection (WFI) can be obtained through filtration by the osmose reverse system, the membrane of which can be disinfected in 18 h contact with an aqueous solution of 1.0% formaldehyde, also followed by rinsing

with a large amount of running water. The 8% formaldehyde alcoholic solution, which has been used, corresponds to a 2.0% formaldehyde aqueous solution, freshly prepared from a 37% (w/v) formaldehyde solution, the industrial distillation of which resulted in a non-intoxicating solution, and a non-irritating vapor.

Furthermore, it is administered as a urinary antiseptic (dialysis [13]), with concentrations in the bladder of 100–200 mg/L [15]. Its releasing agents are also used in the treatment of severe peritonitis.

The disadvantages of formaldehyde is the loss of activity in the presence of organic matters; it is considered potentially cancerigenous [10,13], and may cause eczema and sensitization.

#### **Alcohol (ethanol and isopropanol)**

Besides their broad antimicrobial activity, just as QACs and chlorhexidine, ethanol and isopropanol at 70% are not sporocidal, their effectiveness being due to the inhibition of germinating spores. To reduce populations over 06  $\log_{10}$  of *B. stearothermophilus*, *B. subtilis*, *S. aureus*, *E. cloacae*, and *E. coli*, ethanol and isopropanol have shown a similar average MIC of 8.75% (87500 mg/L). *S. marcescens* and *A. calcoaceticus* were the most sensitive bacteria at a MIC of 4.375% (43750 mg/L). Both alcohols have been largely applied on skin and semi-critical facilities, but they are not used for routine environmental cleaning, and may damage plastic tubes, acrylic and rubber facilities. The presence of 2% glycerin (emollient for hands and forearms) in ethanol at 70% inhibited its activity on germinating spores. The addition of iodine 1% to ethanol at 70% reduced the alcohol MIC (4.375%) to half for *E. coli* and *S. aureus*; and the addition of 10% (w/v) iodine decreased the alcohol MICs to 4.375% and to 2.187% for *B. subtilis* and *E. cloacae*, and for *S. aureus*, respectively.

#### **Comments**

Ethanol is the preferred alcohol in Brazil. It is a by-product of sugar cane fermentation, being very easily encountered and cheap. In São Paulo Hospitals, in the central cleaning-disinfecting area, immersion of non-critical and semi-critical items in ethanol at 70% overnight has replaced phenol and formaldehyde solutions [16] and has been shown to be efficacious.

Alcohol has been added to sanitizing solutions (PVP-I<sub>2</sub>, chlorhexidine, formaldehyde) to prevent contamination with gram-negative bacteria, which are frequently found in hospital environments and can be responsible for the possible formation of a biofilm, hindering the penetration of the disinfectants. Alcohol is also used as a complementary pre-surgical topic antiseptic mixed to PVP-I<sub>2</sub> or

chlorhexidine, the former having a rapid action and the latter, residual action [8]. Trautmann et al. (2001) [17] observed that strains of gram-negative bacteria were not regularly isolated from the hands of hospital personnel, which had been disinfected with alcohol before and after contact with patients.

After appropriate hand washing with disinfectant soap, disinfection of hands by using alcohol (alone or mixed with chlorhexidine or PVP-I<sub>2</sub>) is the best method for preventing outbreaks of infections in health care centers, as well as in food preparation establishments. Conrad (2001) [18] verified that in a 260-bed teaching hospital (Switzerland), the consumption of alcohol increased in the sepsis of hands from 5.7 L hand alcohol per capita per year in 1990 to 9.1 L in 1998, due the introduction of the visualization test to detect the degree of hand disinfection of hospital staff without an increase in skin lesions. For the visualization test, the hands were completely covered with fluorescent alcohol. With a non-germicide lamp, in a dark environment, the areas of the skin which were fully covered, glowed yellow, while the non-covered areas were purple.

#### **Iodine in polyvinyl-pyrrolidone complex (PVP-I<sub>2</sub>)**

For aqueous (topical and soap) PVP-I<sub>2</sub> solutions, the MIC varied from 0.625 to 1.250%. For the alcoholic PVP-I<sub>2</sub> complex, the MIC varied from 0.1560% for *E. coli* to 1.250% for *A. calcoaceticus* and *B. stearothermophilus*. The highest MIC interval of 2.5–5.0 % was for *B. subtilis*, independent of the solution.

#### **Comments**

The incorporation of 10% w/v iodine to the polyvinyl-pyrrolidone (PVP-I<sub>2</sub>) complex strengthens the antibacterial effectiveness and reduces irritation on application. The aqueous PVP-I<sub>2</sub> solution is recommended for topical asepsis; when added with non-ionic detergent (lauryl ether sulfate) it is used as a degerminating solution. The alcoholic PVP-I<sub>2</sub> complex is indicated in delimitation of the surgical site. PVP-I<sub>2</sub> can be used in the absence of chlorhexidine, assuring non allergic cases [8].

#### **Chlorine-Releasing-Agents (CRAs)**

The CRAs, as sodium hypochlorite (NaOCl) and sodium dichloroisocyanurate (NaDCC), are extensively used for antiseptic and disinfecting purposes; and also for decontaminating non-critical surfaces with blood spillage in health care settings [19], despite decreased activity on storage and by organic matter. Similar MIC intervals were observed for the tested bacteria, considering an initial concentration of 8000 – 9000 mg/L free chlorine in NaOCl (pH ≥ 9) and NaDCC (pH 7.0) solutions. The most resistant gram-negative strains, *E. coli* and *A. calcoaceticus*, exhibited a similar MIC (1109.38 – 1497 mg/

L); and for spores, the MIC was between 4000 – 5000 mg/L. On adjusting the pH value to 7.0 of NaOCl solutions, the MIC values were reduced to one tenth, due to the higher predominance of HOCl, the formation of which was observed to stabilize at concentrations ≤ 0.1% CRAs, providing similar MIC (150–300 mg/L) values for the vegetative bacteria and a MIC of 621 mg/L for spores.

#### **Comments**

Sodium hypochlorite (NaOCl) is traditionally used as a base for chlorine disinfectants, exhibiting a wide spectrum antimicrobial activity over a large range of temperatures. It is accessible, easily handled, non-toxic, and compatible with anionic and non-ionic detergents. The undissociated form hypochlorous acid (HOCl) in water at pH 4-7, which is responsible for the microbicidal inactivation by CRAs, has been shown to be 100 times more effective than the dissociated form OCl<sup>-</sup> (pH > 9) [7,10]. Commercial hypochlorite solutions (pH > 8.0) should present a buffering capacity easily favoring dilution to pH 7.0 or lower and accelerating formation of HOCl's strength.

In Brazil, NaOCl and NaDCC solutions at 1% (8000 mg/L of free chlorine) are used for hard-surface disinfection of critical areas in hospitals (intensive therapy unit rooms, nurseries, wards, operating theatres, special procedure ambient) as well as, being added to previously untreated water. NaOCl solutions at 5000 mg/L (0.5%) are allowed to be used in the disinfection of oxygen therapy and anesthesia devices, for overnight exposure [8,16]. NaOCl solutions, stabilized with sodium chloride, may be applied in the disinfection of: (i) nursing bottles (flasks and teats) at concentration of 0.0125% (125 mg/L), (ii) tableware and cutlery, at 0.025% (250 mg/L), (iii) virus contaminated items, at 1.0% (10000 mg/L) [19], (iv) tanks for preparing peritoneal dialysis solutions, at 0.1% (1000 mg/L). Water used to remove residuals of the disinfectants, mainly from those items submitted to a high level disinfection with aldehyde products, must be free of gram-negative bacteria, the residual chlorine concentration being maintained in the range of 0.2 to 0.5 mg/L [8].

#### **Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and per acetic acid (CH<sub>3</sub>COOOH)**

A Brazilian standard H<sub>2</sub>O<sub>2</sub> solution at 4% w/v (40000 mg/L) was able to reduce populations over 08 log<sub>10</sub>, and showed a MIC of 0.125 to 0.25% for *B. subtilis* and *B. stearothermophilus*; a MIC of 0.125 to 0.376% for *E. coli*, *E. cloacae*; a MIC of 0.0625 to 0.0938% for *S. marcescens*, *S. aureus*; and MICs of 0.0313 to 0.0625% for *A. calcoaceticus*.

Peracetic acid (PAA) solution at 3%, in accordance with our data, showed a range of MIC between 0.9 – 1.85 % to reduce populations over  $10^9$  of *B. subtilis* and gram-negative bacteria, and half of that (0.46%) to reduce *S. aureus* and *B. stearothermophilus* populations, after 24 h contact.

#### Comments

Hydrogen peroxide is a powerful oxidizing agent, easily handled and non-toxic, applied on non-critical items. It is a mainstay in metal surface treatment, causing no damage in the disinfection of medical and dental devices in health care routine. It is also used for treating huge volumes of wastewater, and in water and food disinfection applications [8].

Sagripani and Bonifacino (1997) [20] verified an undetectable effect of 25% serum on the sporocidal activity of  $H_2O_2$ , at concentrations lower than 10% (w/v). Because  $H_2O_2$  is not affected by organic matter, the low concentrations used showed good sporocidal activity for 24 h exposure.

Peracetic acid (PAA) is considered a potent biocide at pH 2.0, decomposes to safe by products (acetic acid and oxygen) and remains unaffectedly active in the presence of peroxidases. Nevertheless, Sagripani and Bonifacino (1997) [20] demonstrated that a serum at 5–25% concentration reduced peracetic acid sporocidal activity about 1000 fold, due to the increase of pH to 5.0. PAA had its activity probably partially inhibited by the TSB presented in test tubes.

PAA can be used as a disinfectant of semi-critical medical devices (flexible scopes, hemodialysers) and environmental surfaces. However, it is severely irritating, and can form explosive mixtures with easily oxidized metal substances [8].

Rutala and Weber (1998) [19] remarked that, although the 2% glutaraldehyde-based formulations, 6% stabilized hydrogen peroxide and peracetic acid are among germicides categorized as chemical sterilants, they should be applied after adequate cleaning, following the proper guidelines in regard to organic load, contact time, temperature and pH.

#### Importance of liquid disinfection

At the moment Brazil is being subjected to a strict control of energy consumption. The use of alternative electric equipment, like pasteurizers, has become untenable, because of their high consumption of energy, justifying the wide use of disinfectants which are cheaper and easily acquired and applied.

Another problem, in Brazil at the moment, is water rationing, due to the shortage of rain and the large population in urban areas. Although glutaraldehyde is recommended worldwide [10,19] for the high level disinfection of critical items and formaldehyde for sanitizing water equipment, both demand a large quantity of water for rinsing, in order to remove all residues from the surface of the items. An alternative would be to substitute the aldehydes by other disinfectants or even by associations between chemical agents, which demand less water for rinsing. Therefore, a better strategy would be to first wash the article with enzymatic detergents, thus making the surfaces more permeable, and increasing the action of disinfectants, in general.

#### MIC method appliance

The MIC method allows for comparisons between the microorganisms exposed to the same active chemical agents (biocides); but not between the activity of disinfectants. The presence of soil load, in the form of 5% of bovine serum albumin, represented here by the TSB, is recommended to be added to the bacterial test suspension as a challenge to the chemical agent activity [21]. The limitation of the MIC method is the unknown effect of the broth on each of the disinfectants, even though, the sequential dilution of which in mixture seems to overcome the interference of the broth.

The MIC method shows the range of disinfectant activity over a specific group, genera or strain of bacteria, suggesting the organism which should be used as a biological indicator (BI) to verify the specific chemical activity on a specific application. The MIC method is also used to select a commercial sanitizer containing the same biocide against the same BI. To verify the performance of the disinfectant against an organism on a specific surface, the decimal reduction time (D-value) should be investigated, taking into consideration the resistance of the same organism in direct contact with the biocide.

#### Conclusions

Dilution of the chemical agent to the appropriate bactericidal concentration must be effected with clean potable water. Meanwhile, direct contact with dirty materials should be avoided, the presence of which cause gradual loss of strength and become a vehicle of contamination to other surfaces. Solutions of chemical agents should be kept in closed containers, well protected from air contaminants and provided with a facility which releases a constant required amount.

All over the world, liquid disinfectants are widely employed for their benefit-cost effect. For use in hospitals, the chemical disinfectant should be very easily handled, harmless, and effective for use by nurses caring for chil-

dren, thus preventing cross-infections. The misuse of chemical agents can develop resistant strains of pathogenic microorganisms. Even with the best disinfectants, sterilants, hand washes, and surgical scrubs, cross contamination and infections will occur without proper use of the products. It is essential to adequately control the concentration of diluted products, the contact time of the product, and the cleanliness of the surface prior to disinfection [19]. The tested bacteria are commonly found in the human gastro-intestinal and upper-respiratory tracts, and also in normal skin flora. The correct use of disinfecting agents should be emphasized by educational training and be included in control measures in health care centers, to minimize the risk of cross-infections. The development of hygiene programs standardize the way the chemical agents should be handled.

### Competing Interests section

None declared

### Acknowledgement

We gratefully acknowledge to Brazilian Committees FAPESP and CNPq for the undergraduate scholarships and funds; the assistance of Biologist Irene Machoshvili and Edwards Lifesciences Macchi; and the English revision by Norah Duncan.

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