

Full Length Research Paper

# Determination of decimal reduction time (D-value) of chemical agents used in hospitals for killing airborne isolated bacteria

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Due to the growing numbers of infection outbreaks in hospitals, it is essential to set up an effective sanitation program. The kinetic parameter of killing time (D-value) was experimentally determined to study the effectiveness of some chemical agents used in hospitals. A total of 115 Air-borne bacteria were isolated from 12 wards of a hospital in Alexandria, Egypt. They were tested for their resistance to different antibiotics and commercial disinfecting agents. Three isolates were selected, identified and their D-values were determined against seven chemical agents (hydrogen peroxide, chlorine, betadine, phenik, iodine, dettol, and savlon). The bacterial strains were *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* representing the Gram positive, Gram negative and spore former, respectively. The results revealed that *P. aeruginosa* was the most sensitive organism where *B. subtilis* and *S. aureus* showed approximately same resistance pattern. The data of this study reflect the importance of selecting the appropriate chemical agent to be used for killing specific microorganisms in appropriate time. In conclusion, successful disinfection depends upon the selection of the correct chemical agent associated with a proper disinfecting procedure.

**Key words:** Chemical agents, disinfection, D-value, hospitals, air borne bacteria.

## INTRODUCTION

Antiseptics and disinfectants are freely available without prescription, and are widely used on a daily basis in homes, schools, hospitals, restaurants, farms, abattoirs, other work places and in health care products (Randall et al., 2004), as part of infection control practices and in the prevention of nosocomial infections.

Infections caused by resistant pathogens result in significant morbidity and mortality, and contribute to escalating healthcare costs worldwide. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem throughout the world (Moellering et al., 2007; World Health Organization, 2012b). However, concern is growing worldwide over

uncontrolled use of antiseptics and disinfectants as a result of laboratory data showing a possible link between exposure to these agents and antibiotic resistance (Berlanga and Vinas, 2000; Randall et al., 2004; Russell, 2000). This may be because antiseptics and disinfectants usually have a broad spectrum of activity, multiple non-specific modes of action and usually multiple targets (Moellering et al., 2007). It is important to mention that the environmental impact of successive usage of detergents in hospitals led to the pollution of water bodies (Wyasu and Kure, 2012), thus it is essential to calculate the effective concentration for less environmental impacts. The effectiveness of a disinfectant can be affected

by: (i) previous cleansing of the material; (ii) duration of the application; (iii) concentration of the disinfectant and its final pH; (iv) temperature of the disinfectant during the application (Brazilian Ministry of Health Regulatory Agency, 2003). The effectiveness of a chemical agent can be related to the resistance of a specific microbiological species that can be used as a biological indicator (BI), and can be defined in terms of decimal reduction time (D-value) (Mazzola et al., 2003).

D-Values are defined as decimal reduction times, or the time required to reduce the amount of viable bacteria 1 log<sub>10</sub> or 90% under specified conditions (Jay, 1996). The confidence levels were set for 6 to 12 log<sub>10</sub> reduction of the bacterial population in order to predict probability of the survival microorganism of 10<sup>-1</sup> or better. Jay (1996) early investigated the utility of D-values and acknowledged that this value has meaning in situations where the microbial inactivation kinetics is of 1<sup>st</sup> order. Once the D-value is calculated, it can be used to extrapolate responses beyond the dataset to determine the time required for a desired level of microbial reduction (Mazzola et al., 2003). The application of D-value determination is sometimes used to express antimicrobial efficacy (Mazzola et al., 2003). Few papers reported endemic antimicrobial resistance in Egypt a long time ago, although several reports have studied the occurrence and resistance patterns of specific respiratory and enteric pathogens (Haberberger et al., 1994; Ostroff et al., 1996; Oyofu et al., 1995), and a few small, short-term studies from individual institutions have been reported in Egyptian medical journals (El-Kholy and Nassar, 1996; Samuel et al., 1996). Therefore, in the present study several different currently marketed disinfecting solutions which are commonly used in the Egyptian hospitals, were tested for their efficacy against biocide resistant strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* isolated from hospital air.

## MATERIALS AND METHODS

### Air sampling and microbiological examination

Airborne bacteria were isolated from 12 units of a modern private hospital (Sedi-Aly El Sammak), Alexandria, Egypt, with a total bed capacity of 56 and a total staff of over 200. Isolation was performed by setting prepared nutrient agar plates at a height of the normal human breathing zone (that is 1.5 m above floor level) and exposed to air for about 24 h. After sampling, plates were incubated at 37°C for 48 h. Developed bacterial colonies were purified and cultivated on Blood and MacConkey's agar media. The selected bacterial isolates were characterized and identified according to Microscan Identification System "Dade Behring System".

### Biocides

For this study the following seven biocides were purchased in commercial preparations from local pharmacies.

*Hydrogen peroxide (10%)* is a powerful oxidizing agent, easily handled and non-toxic, applied on non-critical items, widely used for disinfection, sterilization and antiseptic. It is a clear, colorless liquid

commercially available in a variety of concentrations ranging from 3 to 90%. H<sub>2</sub>O<sub>2</sub> demonstrates broad-spectrum efficacy against viruses, bacteria, yeasts and bacterial spores (Mazzola et al., 2003). H<sub>2</sub>O<sub>2</sub> concentrations of 10 to 30% and longer contact times are required for sporidical activity (Gruendeman and Larson, 1998). It is a mainstay in metal surface treatment, causing no damage in the disinfection of medical and dental devices in health care routine (Penna et al., 2001).

Three phenolic compounds were used in this study. Phenol is probably the oldest known disinfectant as it was called carbolic acid. Commercial *Phenik (10%)* solution (pH= 9.0) used in hospitals as disinfectant solution consists of urea, soda, crystal phenol and sulphonic acid (Dvorak, 2005; Jeffrey, 1995). *Dettol (4.8%)* is a phenolic compound composed of 4.8% chloroxylenol, and is commercially available as liquid antiseptic which is safe and gently enough to use on the skin and yet powerful enough as a disinfectant. This is because of its broad spectrum of antimicrobial action. It is effective against Gram positive/negative bacteria, fungi and yeast (Adelowo et al., 2008). *Savlon (3.3%)* consists of 0.3% chlorohexidine and 3.0% cetrimide and is used as disinfectant in worktops, sinks, chopping boards and hospitals. Powerful Savlon liquid is lethal to bacteria (Iroha et al., 2009; Olasehinde et al., 2008).

Chlorine (15%) as sodium hypochlorite (NaOCl) is one of the most widely used products for disinfection and antiseptic purposes. It is very applicable and very effective for the deactivation of pathogenic microorganisms. Chlorine can be easily applied and controlled. It is fairly persistent and relatively cheap to be used as disinfectant for routine cleaning of floors, beds, toilets, walls, rubber draw sheets, and instruments (World Health Organization, 2012a). Chlorine has been used for the disinfection of household areas and for textile bleaching for over more than two hundred years and also used for decontaminating non-critical surfaces with blood spillage in health care settings (Penna et al., 2001).

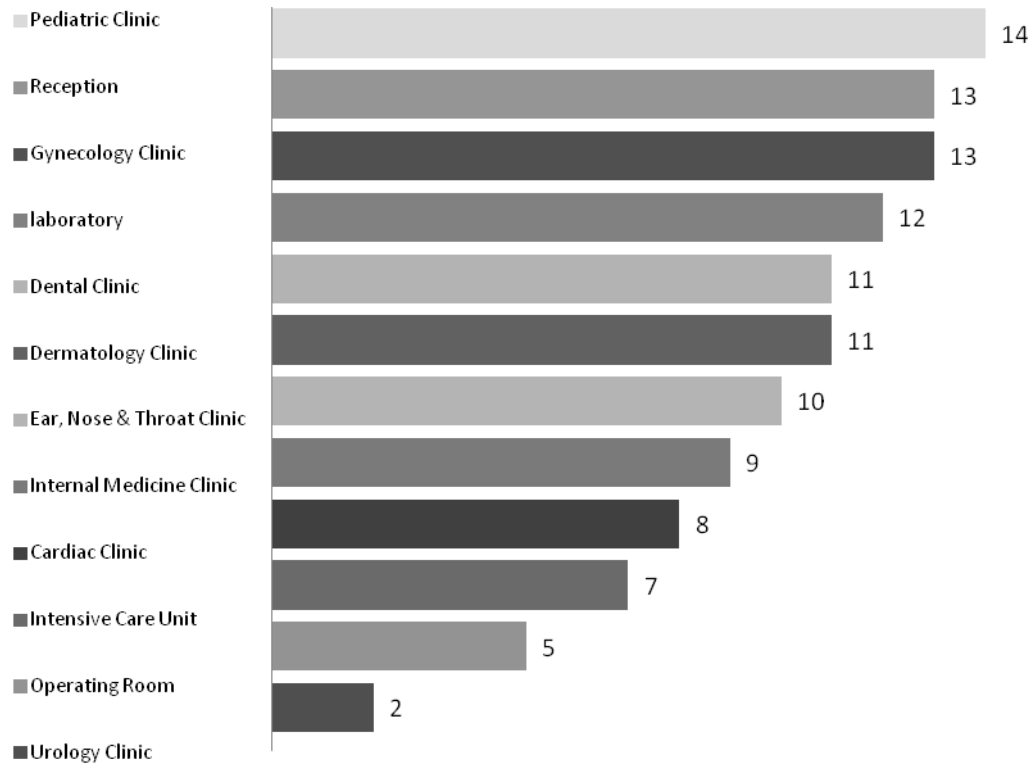
Iodine antiseptics containing povidine-iodine (complex of polyvinylpyrrolidone and iodine), do not negatively affect wound healing, and leave a deposit of active iodine. The great advantage of iodine antiseptics is their wide scope of antimicrobial activity, killing all principal pathogens and, given enough time, even spores, which are considered to be the most difficult form of microorganisms to be inactivated by disinfectants and antiseptics in hospitals (Agerberth and Gudmundsson, 2006). *Betadine (2.5%)* solution contains 10% povidine-iodine solution, used as antiseptics, disinfection, cleaning and de-germing agent for superficial burns.

### Antibiotic resistant profile

The antibiotic resistance profile of bacterial isolates was tested against a number of antibiotics using the Kirby-Bauer disk diffusion method (Hudzicki, 2010). The antibiotics used were: Oxacillin (OX<sub>1</sub>), Vancomycin (VA<sub>30</sub>), Cephadrine (CE<sub>30</sub>), Cefoperazone 75+Sulbactam 30 (CES<sub>105</sub>), Cefadroxil (CFR<sub>30</sub>), Cefaclor (CEC<sub>30</sub>), Cefoperazone (CEP<sub>75</sub>), Ceftriaxone (CRO<sub>30</sub>), Chloramphenicol (C<sub>30</sub>), Erythromycin (E<sub>15</sub>), Trimethprim1.26+Sulfamethoxazole23.7 (SXT<sub>25</sub>) and Rifamycin (RF<sub>30</sub>), representing 7 different groups of antibiotics. The diameter of zone of inhibition formed was measured in millimeters. The antibiotic resistance tests were performed in duplicates.

### Biocides susceptibility testing

The susceptibility of isolated bacteria to the seven commercial disinfectants was determined using the disk diffusion method (Iroha et al., 2009). The test was performed in duplicates using the concentrated form of the chemical agent. The zone of inhibition formed around each disc due to disinfectant effect was measured (Awodele et al., 2007; Iroha et al., 2009). The organism was considered



**Figure 1.** Number of airborne bacteria isolated from different hospital wards.

resistant to a certain biocide when no inhibition zone or inhibition zone less than 10 mm in diameter was observed, whereas, if zone of more than 16 mm was recorded the organism was considered susceptible (Adenike et al., 2011; Okesola and Olola, 2011).

#### Identification of bacteria

Based on susceptibility tests, seven bacterial isolates that showed multiple resistances to biocides and antibiotics were selected for identification. The identification of bacterial species was based on standard laboratory criteria (colony morphology, blood haemolysis, growth on different media and catalase test). For confirmation, the isolates were identified by a fully automated Microscan system (Dade © Behring, Inc. West Sacramento, CA).

#### Assay of D-value

Three bacterial strains representing different groups were selected to study the D-value. They were maintained on tryptic soy agar (TSA, Difo, USA) at 4°C, with monthly transfers. The 24 h cultures grown on tryptic soy broth at 35°C were centrifuged (1000 g / 15 min / 4°C) and suspended in saline (0.95 g/ml NaCl plus 0.1 g/ml peptone) to a final population of 10<sup>6</sup> CFU / ml (Mazzola et al., 2003; Penna et al., 2002). These suspensions were used for the D-value tests against the seven commercial biocides according to the assay of the D-value method described by Mazzola et al. (2003). All biocides susceptibility tests were performed in duplicates.

#### Determination of D-Value and calculation of the confidence level

The D-value at 25°C was determined from the negative reciprocal of the slopes of the regression lines, using the linear portions of the

survivor curves (log<sub>10</sub> CFU/mL versus time of exposure to the disinfectant solution, at constant temperature as described by (Mazzola et al., 2003).

## RESULTS

### Isolation and characterization of airborne bacteria

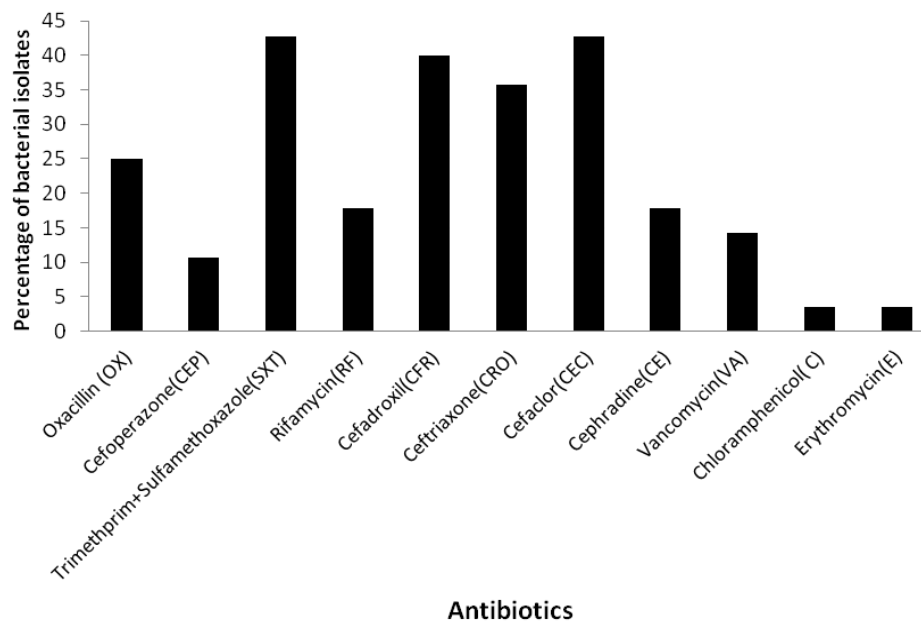
A total of 115 bacterial isolates were recovered from air of the 12 wards in the hospital. The number of airborne bacteria differed according to the ward from which air was sampled. The highest number (14 isolates) was recorded in Pediatric Clinic, followed by 13 isolates obtained in Reception and Gynecology Clinic (Figure 1). The isolated bacteria were purified and subjected to Gram-stain in order to investigate the pattern of distribution of each group. It should be pointed out that in general the Gram-positive colonies comprised 86.5% of the isolates, whereas the Gram-negative formed a fraction of 13.5% only (Table 1).

### Antibiotic resistance profile of bacterial isolates

In this study, none of the isolates showed resistance to CES (Cefoperazone + Sulbactam), whereas approximately 40% of the isolates were resistant to Trimethoprim-Sulfamethoxazole (SXT<sub>25</sub>), Cefaclor (CEC<sub>30</sub>), and Cefadroxil (CFR<sub>30</sub>), and 35% to Ceftriaxone (CRO<sub>30</sub>) (Figure 2). Resistance to Oxacillin (OX<sub>1</sub>) and Rifamycin (RF<sub>30</sub>) was detected in 20% of the isolates, while 10% showed resistance

**Table 1.** Distribution of Gram-positive and Gram-negative bacterial isolates in different hospital wards.

Site	% Gram-Positive Bacteria	% Gram-Negative Bacteria
Laboratory	66.6	33.4
Intensive care unit	57.2	42.8
Operation unit	80	20
Reception clinic	92.3	7.7
Internal medicine clinic	88.9	11.1
Ear, nose and throat clinic	80	20
Dermatology clinic	100	0
Children's clinic	100	0
Urology clinic	100	0
Dental clinic	81.8	18.2
Heart clinic	100	0
Gynecology clinic	92.3	7.7
Total	86.5	13.5

**Figure 2.** Susceptibility pattern of the isolated bacteria to different antibiotics.

to Cephadrine (CE<sub>30</sub>), Vancomycin (VA<sub>30</sub>), Chloramphenicol (C<sub>30</sub>) and Erythromycin (E<sub>15</sub>). In general, the Gram-negative isolates exhibited resistance to antibiotics of seven groups (data not shown). The multidrug resistance phenomenon was observed in this study, since some of bacterial groups showed resistance to cephalosporins 1<sup>st</sup> (Cephadrine and Cefadroxil), 2<sup>nd</sup> (Cefaclor) and third (Cefoperazone and Ceftriaxone) generations as well as Sulphonamide (Trimethoprim-Sulfamethoxazole) and Penicillin (Oxacillin).

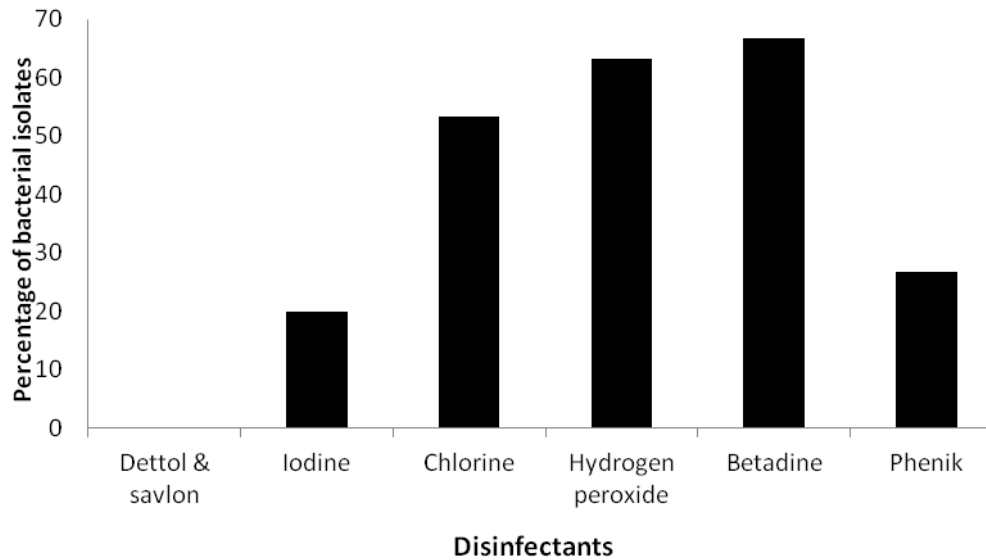
#### Biocide susceptibility pattern of bacterial isolates

Data in Figure 3 show the susceptibility pattern of iso-

lated bacteria to the tested commercial biocides. The highest resistance (66.7% of the total isolates) was recorded towards Betadine followed by 63.3% resistant to 10% Hydrogen peroxide while only 20% of the isolates were resistant to 2.5% Iodine, and none of the examined isolates were resistant to Dettol or Savlon. Thus, susceptibility of biocides was in the following order Dettol and Savlon > Iodine > Phenik > Chlorine > Hydrogen peroxide > Betadine.

#### Identification of bacterial isolates

Isolates showing multiple resistance to antibiotics and disinfectants were selected for identification. Data in



**Figure 3.** Susceptibility pattern of the isolated bacteria to seven concentrated biocides.

**Table 2.** Identification of bacterial strains.

Group	Number of isolates	Identification
Gram positive cocci	11	<i>Micrococcus sp.</i>
	12	<i>Staphylococcus aureus</i>
	17	<i>Staphylococcus xylosus</i>
	22	<i>Staphylococcus simulans</i>
Gram positive bacilli	21	<i>Bacillus subtilis</i>
Gram negative bacilli	8	<i>Pseudomonas aeruginosa</i>
	9	<i>Aeromonas hydrophilia</i>

Table 2 shows that the Gram-positive cocci were represented by one *Micrococcus* sp. and three species of the genus *Staphylococcus* (*S. aureus*, *S. xylosus* and *S. simulans*). One species (*Bacillus subtilis*) belonged to the Gram-positive bacilli, whereas two species; *Pseudomonas aeruginosa* and *Aeromonas hydrophilia* were identified as Gram-negative bacilli. Three bacterial strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*) representing each group were considered as standard biological indicator (BIO) and were selected for further investigation.

#### Determination of D-value and the confidence level

D-values were determined to study the effectiveness of the tested biocides in disinfection processes. The overkill approach to disinfectant agent exposure is based on the premise that the extent of treatment will inactivate the initial bioburden ( $> 10^4$  CFU/mL) and provide an additional safety factor. The D-values and levels of confidence

for every disinfecting solution and bacteria tested are shown in Table 3.

#### Survivor curves

The D-values given in Table 3 were determined from the negative reciprocal of the slopes of the regression lines, using the linear portions of the survivor curves (log<sub>10</sub> CFU/mL as versus time of exposure as shown in Figure 4 a, b, c, d, e, f and g.

In case of H<sub>2</sub>O<sub>2</sub>, the most resistant vegetative strain to 2% solution was *S. aureus* (D = 8.7 min). Due to the sensitivity of *P. aeruginosa* and *B. subtilis* to a 2% H<sub>2</sub>O<sub>2</sub>, they were tested against 0.1%, and showed D values of 3.8, 5.6 min, respectively. A bioburden with 2% H<sub>2</sub>O<sub>2</sub> solution varied between 52.2 min and 104.4 min in relation to *Staphylococcus aureus*, considering high disinfection level. The 6 to 12 log<sub>10</sub> of the sporulation strain *B. subtilis* varied between 33.6 min and 67.2 min, and between 22.8 to 45.6 min for the non-sporulating *P.*

**Table 3.** Decimal reduction times (D-values) for the bacteria in different chemical agent solutions (biocides).

Biocide	Bacteria	Disinfectant Concentration (%)	Survivors Log N	D- value (min)	${}^2t = n * D$ <sup>3</sup> $n = 6$ – log <sub>10</sub> (min)	${}^2t = n * D$ $n = 12$ – log <sub>10</sub> (min)
Hydrogen peroxide 10% pH= 5	<i>Pseudomonas aeruginosa</i>	0.1	-0.258	3.8	22.8	45.6
	<i>Bacillus subtilis</i>	0.1	-0.178	5.6	33.6	67.2
	<i>Staphylococcus aureus</i>	2.0	-0.114	8.7	52.2	104.4
Betadine 10% pH= 6	<i>Pseudomonas aeruginosa</i>	0.01	-0.326	3.0	18.0	36.0
	<i>Bacillus subtilis</i>	1.0	-0.1583	6.3	37.8	75.6
	<i>Staphylococcus aureus</i>	0.1	-0.0984	10.1	60.6	121.2
Phenik 10 % pH= 9	<i>Pseudomonas aeruginosa</i>	0.1	-0.2309	4.3	25.8	51.6
	<i>Bacillus subtilis</i>	0.05	-0.1025	9.7	58.2	116.4
	<i>Staphylococcus aureus</i>	0.1	-0.123	8.1	48.6	97.2
Chlorine 10% pH= 9	<i>Pseudomonas aeruginosa</i>	0.01	-0.223	4.4	26.4	52.8
	<i>Bacillus subtilis</i>	0.5	-0.147	6.8	40.8	81.6
	<i>Staphylococcus aureus</i>	0.1	-0.085	11.7	70.2	140.4
Iodine 2.5 % pH= 5	<i>Pseudomonas aeruginosa</i>	0.0125	0.1293	7.7	46.2	92.4
	<i>Bacillus subtilis</i>	0.25	-0.1489	6.7	40.2	80.4
	<i>Staphylococcus aureus</i>	0.25	-0.245	4.0	24.0	48.0
Dettol 4.8 % pH=7	<i>Pseudomonas aeruginosa</i>	0.0048	-0.1375	7.2	43.2	86.4
	<i>Bacillus subtilis</i>	0.0048	-0.1193	8.3	49.8	99.6
	<i>Staphylococcus aureus</i>	0.0048	-0.125	8.0	48.0	96.0
Savlon 3.3 % pH= 6	<i>Pseudomonas aeruginosa</i>	0.00002	-0.09798	10.2	61.2	122.4
	<i>Bacillus subtilis</i>	0.00002	-0.1022	9.7	58.2	116.4
	<i>Staphylococcus aureus</i>	0.00002	-0.1514	6.6	39.6	79.2

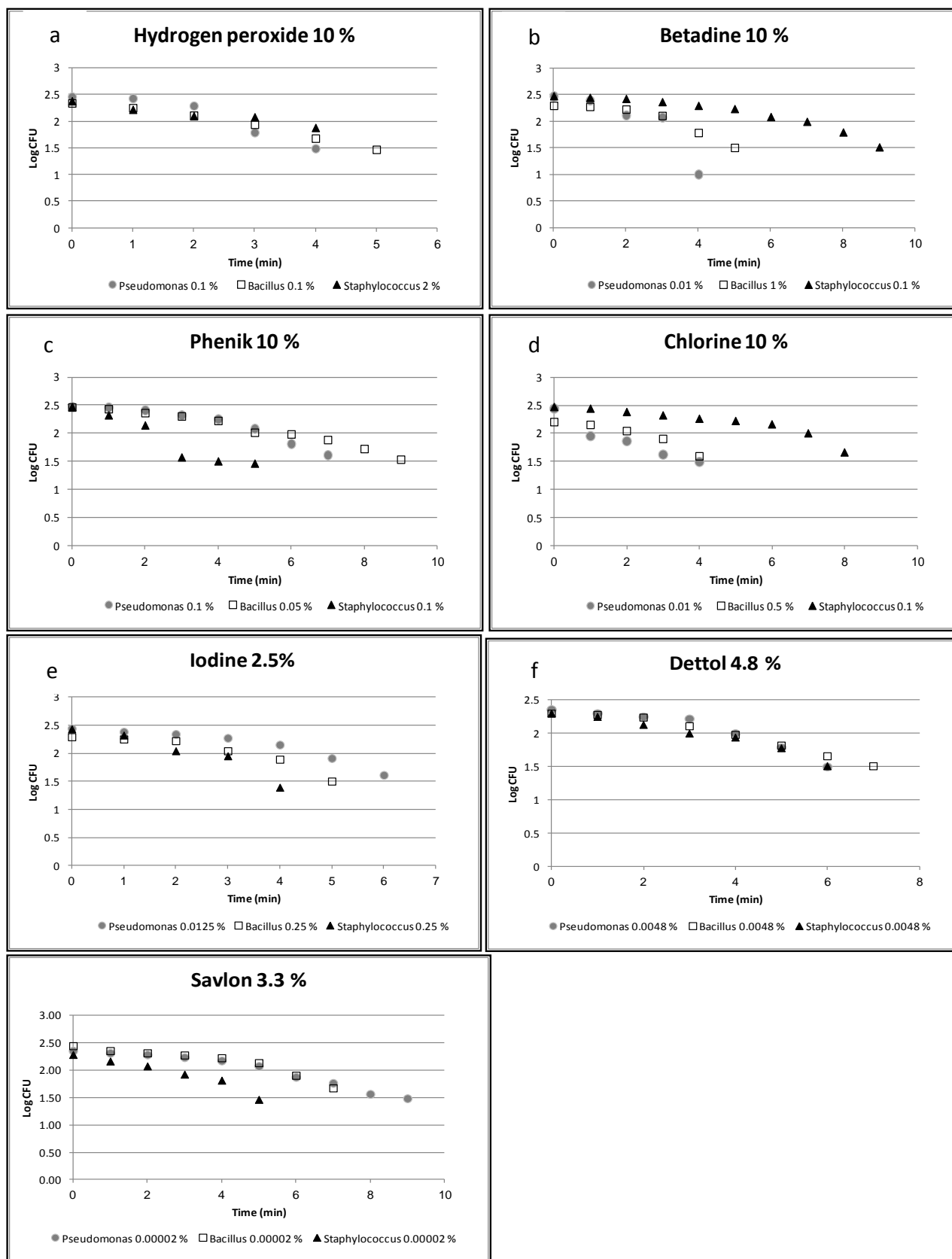
*aeruginosa*, considering low level disinfection with 0.1 % H<sub>2</sub>O<sub>2</sub> solution (Table 3).

The vegetative strain which showed best resistance to 1% Betadine solution was *B. subtilis* (D = 6.3 min), followed by *S. aureus* against 0.1 % Betadine (D = 10.1 min) whereas, *P. aeruginosa* was the most sensitive to 0.01 % Betadine solution (D= 3.0 min) (Table 3).

The D-values for *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 8.1 min and 4.3 min respectively when exposed to 0.1% Phenik, whereas a value of 9.7 min was

recorded for *Bacillus subtilis* when exposed to a 0.5% Phenik solution being the most sensitive bacterium (Table 3).

*B. subtilis* was the most resistant bacterium to chlorine exhibiting a decimal reduction time (D= 6.8 min) at 0.5% concentration of commercial chlorine solution 10%. On the other hand, the most sensitive strain was *P. aeruginosa* with D = 4.4 min after exposure to 0.01% of chlorine solution. While *S. aureus* showed decimal reduction time D = 11.7 min when exposed to 0.1% chlorine



**Figure 4.** Survivor curves showing the reduction of *P. aeruginosa*, *B. subtilis* and *S. aureus* isolates exposed to different commercial biocides (a) Hydrogen peroxide; (b) Betadine; (c) Phenik; (d) Chlorine; (e) Iodine; (f) Dettol and (g) Savlon.

solution.

The D-values observed for vegetative bacteria in a 0.25% dilution of 2.5 % iodine solution were *B. subtilis* (D= 6.7 min) followed by *S. aureus* (D= 4.0 min), but *P. aeruginosa* was killed after the first min exposure to the same concentration. A D-value of 7.7 min was recorded when *P. aeruginosa* was exposed to a 0.0125 % concentration.

As shown in Table 3, when Dettol was applied at 0.0048% concentration, the reduction time was almost the same for the tested bacterial strains (D = 7.2, 8.3 and 8.0 for *P. aeruginosa*; *B. subtilis* and *S. aureus*), respectively.

In a solution of Savlon the decimal reduction values of the spore former *B. subtilis* and non-spore former *P. aeruginosa* were similar (10.2 and 9.7 min, respectively) when diluted Savlon (0.0002%) was used. *S. aureus* showed more sensitivity with a decimal reduction time of 6.6 min.

## DISCUSSION

Hospitals provide reservoirs of multi-resistant microorganisms borne by patients and staff. Preventing the spread of relevant bacteria depends on the quality of hospital routine cleaning services. Monitoring the bacteria susceptibility to antimicrobials and disinfectants may help the management of nosocomial infections (Bouzada et al., 2010).

The number of bacteria isolated in this study is in good agreement with the observation of Ekhaie et al. (2010), Hudzicki (2010) and Kim et al. (2010) for bacteria recorded in private and government owned hospitals in Nigeria and Korea. The dominance of Gram-positive group observed in this study is similar to data previously reported in a hospital in Korea (Kim et al., 2010), and in clinical isolates as well (Adelowo et al., 2008; Langsrud et al., 2003).

Microbial resistance to antimicrobials has been frequently associated to indiscriminate use of antibiotics, therapeutic or prophylactic, emphasizing the fact that scientific criteria are not respected in the prescription of these medicines. Rather, a rational use of antibiotics should be exercised in order to prevent selective pressure originated by indiscriminate use of these compounds. According to the literature, the high level of antimicrobial resistance to drugs used in hospitals and in the community constitutes an important alert to this severe phenomenon, which is considered one of the great challenges to science and medicine in the 21st century (ASM, 2009).

In this study, antimicrobial resistance was observed between isolates and the degree of resistance varied according to bacterial strain. Multidrug resistance can be defined as bacterial resistance to 3 or more different classes of antibiotics, a phenomenon known to occur within Gram-positive (Cerf et al., 2010) and Gram-negative (Davina-Regli et al., 2008) bacteria. The multidrug re-

resistance observed in this study was previously demonstrated by (Prinsloo et al., 2008). The high levels of resistance against, Trimethoprim- Sulfamethoxazole to Cefaclor, Cefadroxil and Ceftriaxone are relevant since these antimicrobials are used in the hospitals and in the community. However, the low levels of resistance to Vancomycin, Chloramphenicol, Cephadrine and Erythromycin might indicate that these compounds are carefully controlled in our health system.

The susceptibility pattern obtained for the isolated bacteria towards 7 different commercial antimicrobial agents was in the following order: Dettol and Savlon > Iodine > Phenik > Chlorine > Hydrogen peroxide > Betadine. The high susceptibility to Savlon was recorded previously by (Iroha et al., 2009; Saha et al., 2009) who showed that Savlon has appreciable antimicrobial activity against clinical isolates. The phenomenon of selectable biocide resistance has been early demonstrated by (Braoudaki and Hilton, 2004a, 2004b; Ledger et al., 2006; Stickler and Jones, 2008). However, most of the evidence on bacterial resistance to biocides comes from laboratory-based experiments which investigated a wide range of agents such as cationic biocides (Thomas et al., 2000), isothiazolones (Winder et al., 2000), phenolics (McMurry et al., 1999), hydrogen peroxide and peracetic acid (Dukan and Touati, 1996). Attention should be given to the resistance strains to sodium hypochlorite. This substance is widely used in hospitals. It is a fast reactant of low cost and should be indicated for medium level disinfection of articles and surfaces, for 10 min in concentrations ranging from 0.2 to 1% (Bouzada et al., 2010; Kramer et al., 2006; Rossi et al., 2008; Rutala and Weber, 2007).

Identifying microorganisms and their susceptibility patterns to antimicrobial drugs and nosocomial disinfectants could be useful to trace origins and determine the persistence of bacteria potentially associated to hospital infections. Therefore, the isolates showing multiple resistances to antibiotics and disinfectants were selected for identification. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were considered biological indicators (BIO). As reported by Dutkiewicz and Augustowska (2006), the indoor environment can potentially place human occupants at greater health risk. Bacterial species isolated in this study were previously encountered in hospital infections (Ekhaie et al., 2010; Kim et al., 2010).

D-values must be determined for classification and studying the effectiveness of the chemical agents in disinfection processes, to be used in the hospitals control program for health care environment (Adenike et al., 2011; Hudzicki, 2010). However, there is no disinfectant that can serve all situations. The overkill approach to disinfectant agent exposure is based on the premise that the extent of treatment will inactivate the initial bioburden (> 10<sup>4</sup> CFU/mL) and provide an additional safety factor (Agerberth and Gudmundsson, 2006; Hudzicki, 2010). In general, a disinfectant is expected to be capable of at



least a 5-log<sub>10</sub> reduction of pathogenic bacteria during a time frame greater than 5 but lower than 10 minutes (Rutala, 1995). However, the elimination of spores requires longer exposure time to attain the confidence level established (Mazzola et al., 2003). Our study showed that in general the opportunistic pathogen *Pseudomonas aeruginosa* showed the highest sensitivity against the tested biocides followed by the Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and this agrees with the results of (Olasehinde et al., 2008) who found that biocides had broad activity against *P. aeruginosa*, *Proteus mirabilis* and *Salmonella typhi*. *Pseudomonas aeruginosa* was sensitive to chlorine and this come in accordance with (Mazzola et al., 2006) who found that *Pseudomonas* was the most sensitive organism to chlorine. On the other hand, *Pseudomonas aeruginosa* tested showed less sensitivity to Savlon and this agrees with Olasehinde et al. (2008) who found that Savlon showed less activity against *Pseudomonas*. The lower D-values were recorded to Dettol and Savlon which confirmed results obtained by Adelowo et al. (2008) and Olasehinde et al. (2008) that they have broad spectrum activity. They were the best killers for the three different strains but due to their expensive prices either iodine or chlorine can be used for *Pseudomonas aeruginosa*, hydrogen peroxide and phenik for *Bacillus subtilis* and phenik for *Staphylococcus aureus*.

The foregoing work states that the bacterial suspensions studied were an indication of the disinfectant efficacy on a surface, and surface testing is widely recommended by regulatory standards (Adelowo et al., 2008; Brazilian Ministry of Health Regulatory Agency, 2003; Okesola and Olola, 2011; Penna et al., 2002). Therefore, the data in this study reflect the formulations used, which may vary from product to product. The contact time and the dilution of disinfectant varied according to the micro-organism which needed to be killed, thus it should be reviewed to reach the sufficient bioburden reduction time (over 6 log<sub>10</sub>). This study is one of the studies that give an alarm for excess using the biocides without any care and precautions that will lead to increase the resistance organism instead of getting rid of them.

## Conclusion

Dilution of the chemical agent to the appropriate bactericidal concentration and exposure time to the contaminant needed to be killed are the limiting factors for disinfection processes. It is also essential that the chemicals used in commercial products and in the preparation of the disinfecting solutions meet established quality requirements.

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