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Full Length Research Paper

Effect of liquid disinfectant Morigad® on the growth patterns of *Staphylococcus aureus, Escherichia coli* and *Candida albicans*

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The antimicrobial properties of various use dilutions of Morigad® against some test and control strains of nosocomial infectious agents namely *Escherichia coli, Staphylococcus aureus* and *Candida albicans* were investigated by measurement of viability of the organisms using turbidometric studies and various diluents (sterile tap water (STW), sterile deionized water (SDW) and 10% rabbit serum) and cultivation on solid media. Results showed that loss of viability was faster in SDW, followed by STW, then 10% rabbit serum. A higher percentage of the resistant (control) strains S_1, E_1 and C_1 survived compared to the susceptible (test) strains S_2, E_2 and C_2. The viability of the cells in the disinfectant was also concentration dependent with the death rates ($Kmin^{-1}$) higher in SDW than in STW and *E. coli* more susceptible followed by *S. aureus* and *C. albicans.* Measurement of microbial resistance using decimal reduction time (DRT) against the lethal effects of the disinfectant shows that the slopes were lower for the test organisms and higher for the control organisms, while *E. coli* (E_1 and E_2) had higher DRT, followed by *S. aureus* (S_1 and S_2) and then *C. albicans* (C_1 and C_2). Result also showed that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values from the different diluents used were highest for serum, followed by STW, while the lowest values were obtained from SDW. For the organisms investigated, *E. coli* had the highest MIC (0.042) and MBC (0.046) followed by *S. aureus* (MIC 0.034, MBC 0.038), while *C. albicans* demonstrated the lowest MIC (0.032) and MBC (0.034) values.

Key words: Antimicrobial resistance, disinfectant, Morigad®, MIC, MBC, nosocomial infection, viability, turbidometry.

INTRODUCTION

Nosocomial infection is an infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission (WHO, 2002). These include infections acquired in the hospital but appearing after discharge and occupational infections among staff of the hospital or healthcare facility. Nosocomial infection affect 5% of all hospitalized patients and in some clinical services such as intensive care units, up to 10% of the patients are infected in the developing countries (Madigan et al., 2000).Reviewing the incidences and spread of nosocomial infections, Lark (2000) observed that about patients in the United States developed bacteraemia or fungemia each year, with an associated mortality of 12- 50% per episode. Unlike other fungal apthogens, *C. albicans are* part of the normal hu-man flora and are commonly found on the skin and throughout the gastrointestinal tract (GIT). These endemic dimorphic fungi grow as saprophytic moulds in the environment, but at 37°C transform into parasitic yeast forms well adapted for survival in mammalian hosts. *C. albicans invade* through the GIT and skin. Only a relatively small number of organisms cause nosocomial infec-tions such as *Candida*, *Staphylococcus*, *Pneumococcus*, *Pseudomonas* and *Escherichia* species (Lark, 2001). Similar incidences have been reported in Europe, Japan, South-East Asia, the Middle East and Australia (Brown et al., 2003; Matsuda et al., 2003; Yan et al., 2003; Fidler, 2003). The most frequent

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infections are those of surgical sites as well as skin and soft tissue sites, blood, urinary, upper and lower respiratory tract infections. Most of these infections are associated with invasive medical devices or invasive surgical procedures (Loutfy et al., 2004). Factors facilitating spread of nosocomial infections are impaired immunity, extremities of age, severe illnesses, treatments with broad spectrum antibiotics, the ever increasing variety of medical procedures and invasive techniques creating potential routes of infection (e.g. those patients who have open wounds or tube going into their body) and transmission of drug resistant microorganisms among crowded hospital populations, where poor infection control practices may facilitate transmission (Amita et al., 2003).

The impact of nosocomial infection on an individual or community are frequent visits and greater length of hospital stay, high rates of illness, loss of productivity and death, straining of family and community budgets and extra time of hospital staff. At national levels, nosocomal infections diverts financial resources that that could otherwise be used for improving health and threatens the success of global efforts to combat the major infectious diseases of poverty and ignorance. Even in developed countries, measures to combat the emergence and spread of resistant nosocomial pathogens have met with varying degrees of success (Schwaber et al., 2004). Given that only a few number of disinfectants are available in developing countries because of limited resources or cost restrictions, the surveillance of nosocomial pathogens and proper use of whatever disinfectants and other antimicrobial agents available cannot be overemphasized. Morigad® germicide is a liquid disinfectant frequently used in slaughter houses, hospitals and other healthcare settings in Adamawa State of Nigeria constituted in various dilutions. Quite often, studies to determine the efficacies of antiseptics and disinfectants utilize organisms that have little relevance to the hospital environment (Elmahmood, 2006). In the clinical setting, microorganisms are not found in pure cultures but enveloped in proteinaceous materials such as blood, sputum, feces or milk. Similarly, distilled water regularly used in the investigations, is not the water that is used in practice to reconstitute the disinfectants (Elmahmood, 2006). The objective of this study is therefore to evaluate the efficacy of Morigad® disinfectant under use-conditions against some microorganisms implicated in causing nosocomial infections, notably, *S. aureus, E. coli* and *C. albicans.*

MATERIALS AND METHODS

Data collection using questionnaires

The selection of microorganisms was based on analysis of questionnaires previously issued to hospital personnel to determine the diseased conditions that predispose patients to nosocomial infections, the most frequent organism causing nosocomial infections, the antibiotics frequently prescribed and the most frequently used disinfectants at the Federal Medical Center, Yola, Nigeria in order of importance. The selected organisms were *S. aureus, E. coli* and *C. albicans,* while the selected disinfectant was Morigad® germicide.

Selection of patients for sample collection

Based on responses obtained from the questionnaires, patients with urinary and respiratory tract, septicemia, catheter and other indwelling devices, skin and soft tissue infections as well as surgical patients were considered possible cases. Immediately after admission, these patients were screened for the presence of any of the selected organisms and any positive cases ruled out for the purpose of this study. Those that did not show the presence of the selected organisms were considered as possible cases for further observation of their clinical conditions. For the purpose of specimen collection, patients were classified into cases and controls. Specimens including urine, stool, sputum, wound exudates, saliva and blood were then collected from these patients after their appropriate hospital stay and after 72 h hospitalization. It was from some of these patients that the selected organisms were isolated (Schwaber et al., 2004; Scherer et al., 2005).

Isolation and identification of microorganisms

This was carried out as described by Cheesbrough (2002). A nosocomial isolate was defined as an isolate obtained from a site considered to be infected by the treating Medical Doctor.

Selection of test and control organisms

The isolated bacteria (*S. aureus* and *E. coli*) and fungi (*C. albicans*) were subjected to antimicrobial susceptibility testing using the disc diffusion method described by Gupta et al. (2004) for bacteria and Archibald et al. (2004) for fungi. Based on the susceptibility results obtained, the organisms were grouped into resistant (*S. aureus* S_1, *E. coli* E_1 and *C. albicans* C_2) and susceptible (*S. aureus* S_2, *E. coli* E and *C. albicans* C_2). Resistant organisms were those that showed stable resistance to more than 3 antimicrobial agents and susceptible organisms were those that showed stable susceptibility to all the drugs tested (Gupta et al., 2004; Achibald et al., 2004).

Sources of disinfectant, media and antibiotic discs

Morigad® germicide (5 L gallon) (Morison Industries Plc, Ikeja, Lagos, Nigeria) was purchased from Mahmuda Pharmaceutical Chemist Shop Yola, Adamawa State, Nigeria. Chemically, it consisted of Dichloroxylenol plus chlorophenol and phenol 32% v/v. The manufacturer's recommended use dilutions were 1:100 (0.01 v/v), 1:200 (0.0033 v/v), 1:400 (0.0025 v/v) and 1:600 (0.0017 v/v). All media and suspending media used were of Oxoid grade and the antibiotics were of Optun products obtained commercially. The antifungal drugs used for the susceptibility tests were nystatin, itraconazole, miconazole, fluconazole and ketoconazole purchased from Mahmud Pharmacy, Yola, Adamawa State, Nigeria.

Maintenance of the test organisms

The selected test and control organisms were sub cultured on nutrient agar slants and stored at 4°C until required. The purity of the organisms was checked at regular intervals by plating and staining (Acheampong et al., 1988).

Preparation of cell cultures

The calibration of the organisms was carried out by following

changes in optical density and in viable cell count for a period of 120 min (Acheampong et al., 1988). Cell cultures of the selected organisms in nutrient broth were grown in a shaker water bath maintained at 37°C to obtain absorbance values of:

a.) 0.52 for S_1 and 0.438 for S_2, 0.51 for E_1 and 0.43 for E_2, 0.45 for C_1 and 0.475 for C_2 that corresponded to 2.0 x 10^8 cellsml^{-1} (culture A).
b.) 0.45 for S_1 and 0.37 for S_2, 0.395 for E_1 and 0.387 for E_2, 0.41 for C_1 and 0.40 for C_2 that corresponded to approximately 1.0 x 10^8 cellsml^{-1} (culture B). Part of culture B was diluted with sterile nutrient broth to produce 1.0 x 10^4 cellsml^{-1} (culture C). Cultures A, B and C were stored at 4°C for further experiment.

Effect of 1:100 (0.01 v/v) of Morigad® on the viability of the test organisms (S_1, S_2, E_1, E_2, C_1 and C_2)

To 0.2 ml of undiluted Morigad® solution, 17.8 ml sterile deionized water (SDW) was added in a 50 ml conical flask and 2 ml of culture A (S_1) added and the flask shaken vigorously. 1 ml of the suspension was then immediately transferred into a test tube containing 9 ml inactivator solution of 2% tween 80 plus 1% soy lecithin to inactivate the dichloroxylenol and chlorophenol and other phenolic contents of the Morigad®. This was then adequately mixed on a gallenkamp whirl mixer and allowed to stand for 1 min for complete inactivation2 of the disinfectant (Ray et al., 1968; Russel et al., 1979; Acheampong et al., 1988).

Subsequent dilutions of the cell suspension were made in 9 ml trypton soy broth (recovery medium) and 1 ml of the final dilution was cultured using the pour plate technique at 0 and 5 min intervals for 30 min. The cultures were incubated at 37°C for 24 h and colonies counted using the Qubec Darkfield Colony Counter. The viability of the untreated (control) organisms was also determined under similar conditions. Graph of log N_t/N_o versus time were plotted. The same procedure was repeated for each of the other five organisms. For the 1:100 (0.01 v/v) use-dilution of Morigad® in STW, the same procedure as for the SDW diluent was also followed except that sterile tap water (STW) was added in place of SDW.

Effect of 1:300 (0.0033 v/v) of Morigad on the viability of the test organisms (S_1, S_2, E_1, E_2, C_1 and C_2)

Sterile distilled water (SDW) (17.93 ml) was added to 0.067 ml of undiluted Morigad® in a 50 ml conical flask and properly swirled to mix. 2 ml of culture A was then added and mixed on a gallenkamp whirl mixer and allowed to stand for 1 min for complete inactivation of the disinfectant. To determine the effect of the disinfectant on the viability of the test organisms the same procedure previously described for 1:100 dilution was also repeated for all the five organisms.

Effect of 1:400 (0.0025 v/v) of Morigad on the viability of the test organisms (S_1, S_2, E_1, E_2, C_1 and C_2)

For this dilution, 0.05 ml of undiluted solution of the disinfectant was added to 17.95 ml of SDW and 2 ml of culture A added and mixed as earlier described. To determine the viability of the five test organisms and also for the effect of 1:400 dilution in STW, earlier described procedure was also followed except that STW was added in place of SDW.

Effect of 1:600 (0.0017 v/v) of Morigad on the viability of the test organisms (S_1, S_2, E_1, E_2, C_1 and C_2)

To 17.967 of SDW in a sterile conical flask, 0.033 ml of undiluted solution of Morigad® and 2 ml of culture A (S_1) and thoroughly swirled to properly mixed the contents. To determine the viability of the test organisms in this dilution, the procedure earlier described for 1:100 dilution (0.01 v/v) was followed and was repeated for the 5 other test organisms as well as for use dilution of 1:600 (0.0017 v/v) in STW except that in this case STW was added in stead of SDW.

Determination of MIC of Morigad® in SDW, STW and 10% rabbit serum using culture B (1.0 x 10^8 cellsml^{-1})

This carried out using the logarithmic dilution method as described by Croshaw (1983). Graded volumes of freshly prepared 1:100 (0.01 v/v) dilution of Morigad® (1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8 and 3.0 ml) and respective calculated volumes of SDW and STW (3.8, 3.6, 3.4, 3.2, 3.0, 2.8, 2.6, 2.4, 2.2, 2.0, 1.8, 1.6, 1.4 and 1.2 ml) were added to respective 5 ml double strength nutrient broth and 0.2 ml of culture B (11.0 x 10^8 cellsml^{-1}) added to make a total volume of 10 ml in single strength nutrient broth and cell density of 1.0 x 10^7 cellsml^{-1}. This mixture was thoroughly mixed on a whirl mixer. The 11th test tube did not contain Morigad® and served as control. The test tubes were then incubated at 37°C for 24 h and observed for growth in form of turbidity. The same procedure was repeated for the rest of the 5 test organisms. The viability of the test organisms was also studied by repeating the above procedure against all the test organisms but this time by adding 1 ml rabbit serum to the content of the test tubes so as to make a 10% serum concentration in each case of SDW and STW dilutions.

Determination of MBC of Morigad® in SDW, STW and 10% rabbit serum using culture B

Two loopfuls of broth culture was removed from the test tubes that did not show any turbidity in the MIC determination after incubation at 37°C for 24 h and inoculated on nutrient agar for *S. aureus* (S_1 and $_2$) and *E. coli* (E_1 and $_2$) and Saboroud Dextrose Agar for *C. albicans* (C_1 and $_2$). The plates were then incubated at 37°C for 24 h and observed for growth (Waterworth, 1978 and Baldry, 1984).

Determination of MIC of Morigad® in SDW, STW and 10% rabbit serum using culture C (1.0 x 10^4 cellsml^{-1}). Graded volumes of freshly prepare 1:100 (0.01 v/v) use-dilutions of Morigad® (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4 and 3.6) and calculated volumes of SDW (4.4, 4.2, 4.0, 3.8, 3.6, 3.4, 3.2, 3.0, 2.8, 2.6, 2.4, 2.2, 2.0, 1.8, 1.6, 1.4 and 1.2) were added to respective 5 ml double strength nutrient broth and 0.2 ml of culture C (1.0 x 10^4 cellsml^{-1}) of S_1 added to make up to 10 ml in single strength nutrient broth and cell density of 1.0 x 10^3 cellsml^{-1} and MIC was determined as earlier described for culture B. The procedure was repeated for the other 5 test organisms as well as for STW and 10% rabbit serum except that STW and 10% rabbit serum were used in place of SDW.Determination of MBC of Morigad® in SDW, STW and 10% rabbit serum using culture C (1.0 x 10^4 cellsml^{-1}). To determine the MBC for culture C the same procedure as earlier described for culture B was repeated but this time using culture C (1.0 x 10^4 cellsml^{-1}) in stead of Culture B.

RESULTS

The proportion of cells that survived just after 10 and 30 min of contact with use dilutions of Morigad® are shown in Table 1. Results showed that for 1:600 (0.0017 v/v) use dilutions of Morigad®, 5.4% of cells of *S. aureus* (S_1) survived in SDW after 10 min and decreased to 0.200% after 30 min of contact, while in STW for S_1, 7.0% of cells survived after 10 min but further decreased to 0.001%

Table 1. Determination of number of viable cells after 10 and 30 min of treatment with different recommended use dilutions of disinfectant Morigard® (chloroxylenol plus chlorophenol).

Conc v/v	S_1				S_2				E_1				E_2				C_1				C_2			
	10 min		30 min		10 min		30 min		10 min		30 min		10 min		30 min		10 min		30 min		10 min		30 min	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0.1 SDW	1.9x 10^6	1.0	6.0 x 10^3	0.003	1.3x 10^6	0.70	4.7 x 10^3	0.002	1.6 x10^6	0.8	7.9 x 10^3	0.004	1.9x 10^6	0.9	5.7 x 10^3	0.003	1.2x 10^5	0.5	4.5 x 10^3	0.002	5.1 x 10^5	0.3	2.0 x 10^3	0.001
		1.2		0.008		0.9		0.004		1.1		0.008		1.3		0.004		0.7		0.003		0.4		0.001
STW	2.5x 10^6		1.6 x 10^4		1.8x 10^6		8.4 x 10^3		3.3x 10^6		1.7 x 10^4		2.5x 10^6		8.7 x 10^3		1.3x 10^6		6.5 x 10^3		8.3 x 10^5		2.9 x 10^3	
0.0033SDW	3.2x 10^6	1.6	2.9 x 10^4	0.020	2.4x 10^6	1.2	1.3 x 10^4	0.007	4.1x 10^6	2.0	2.5 x 10^4	0.010	2.9x 10^6	1.5	1.1 x 10^4	0.005	1.8x 10^6	0.9	1.1 x 10^4	0.005	1.0 x 10^6	0.5	8.1 x 10^3	0.004
		2.5		0.030		1.4		0.010		2.3		0.020		2.2		0.010		1.1		0.008		0.8		0.006
STW	5.0x 10^6		5.5 x 10^4		2.8x 10^6		2.4 x 10^4		4.7x 10^6		3.6 x 10^4		4.4x 10^6		2.4 x 10^4		2.2x 10^6		1.7 x 10^4		1.7 x 10^6		1.2 x 10^4	
0.025 SDW	6.7x 10^6	3.3	7.1 x 10^4	0.040	4.2x 10^6	2.1	3.3 x 10^4	0.020	8.0x 10^6	4.0	7.1 x 10^4	0.040	5.2x 10^6	2.6	3.2 x 10^4	0.020	3.6x 10^6	1.8	1.9 x 10^4	0.010	1.8 x 10^6	0.9	1.9 x 10^4	0.009
		4.6		0.060		2.8		0.040		5.0		0.050		3.7		0.030		2.2		0.020		1.7		0.010
STW	9.1x 10^6		1.2 x 10^5		5.6x 10^6		7.3 x 10^4		1.0x 10^7		8.9 x 10^4		7.3x 10^6		6.8 x 10^4		4.5x 10^6		4.3 x 10^4		3.5 x 10^6		2.3 x 10^4	
0.0017SDW	1.1 x10^7	5.4	1.9 x 10^5	0.200	9.1x 10^6	4.6	9.2 x 10^4	0.050	1.3x 10^7	6.4	1.8 x 10^5	0.090	8.6x 10^6	4.3	9.5 x 10^4	0.050	6.6x 10^6	3.3	4.5 x 10^3	0.002	4.1 x 10^6	3.5	4.3 x 10^4	0.020
		7.0		0.100		7.0		0.090		9.5		0.200		4.8		0.060		3.8		0.003		2.0		0.040
STW	1.4 x10^7		3.1 x 10^5		1.4x 10^7		1.8 x 10^5		1.9x 10^7		4.7 x 10^5		9.5x 10^6		1.2 x 10^5		7.6x 10^6		6.5 x 10^3		6.9 x 10^6		7.3 x 10^4	

N = Number of viable cells; % = Percentage of number of viable cells; S_1 = *S. aureus*; S_2 = *S. aureus*; E_1 = *E. coli*; E_2 = *E. coli*; C_1 = *C. albicans*; C_2 = *C. albicans;* SDW = sterile deionized water and STW = sterile tap water.

after 30 min of exposure. For the lower use dilution of 1:100 (0.001 v/v) for *S. aureus* (S_1), the percentage of viable cells in SDW after 10 min was 1.0 and 0.003% after 30 min, while in STW, the percentage of cells of S_1 was 1.2% after 10 min and 0.008% after 30 min. For the control strain of *S. aureus* (S_2) using 1:600 (0.00117 v/v) in SDW, the percentage of cells decreased to 4.6% after 10 min and 0.05% after 30 min. In SDW, the percentage of S_2 decreased to 7.0% after 10 min and further more to 0.09% after 30 min for the 1:600 (0.0017 v/v) use dilution. In SDW, the percentage of viable cells of S_1 decreased to 0.9% after 10 min and to 0.004% after 30 min of exposure to the lower use dilution of the disinfectant. This pattern of loss of viability was similar for all the other four test organisms with a decline in viability in SDW than in STW. A higher percentage of the resistant strains (S_1, E_1 and C_1) of the cells also survived compared to the susceptible cells (S_2, E_2 and C_2) of the test organisms.

The effect of the use dilutions of Morigad® on the viability of the organisms are shown in Figure 1a (*S. aureus* S_1), Figure 1b (*S. aureus* S_2), Figure 2a (*E. coli* E_1), Figure 2b (*E. coli* E_2), Figure 3a (*C. albicans* C_1) and Figure 3b (*E. coli* C_2). The graphs were plotted as log N_t/N_o versus time from the data in Table 1. Extrapolation of the graphs to the log N_t/N_o axis gives the log extrapolation numbers. The curves initially showed a lag, the duration of which depended on the concentration of the disinfectant used, the type of water diluent and organisms before the exponential order of death as shown by the almost straight line graphs.

The death rates ($Kmin^{-1}$) of the organisms obtained from the graphs are given in Table 2. Results showed that at the higher use dilution of 1:600 (0.0017 v/v), the death rate, $Kmin^{-1}$ for S_1 was -0.18 in SDW and -0.16 in STW indicating that the death rate was faster in SDW than in STW. Similarly, for the lower use dilution of 1:100 (0.01 v/v) for S. *aureus* (S_1), the death rate $Kmin^{-1}$ was -0.28 in SDW and -0.25 in STW, while for S_2, the death rates were -0.30 in SDW and -0.29 in STW. *S. aureus* (S_2) had a death rate of -0.23 in SDW and -0.22 in STW. For all the organisms studied, the death rates were higher in SDW than in STW. *E. coli* had lower death rates, followed by *S. aureus*, while *C. albicans had* the lowest death rates. For all the organisms, the death rates were faster for the control than the corresponding test organisms. Values of the Decimal Reduction Time (DRT) and the Slopes (M) of the graphs are

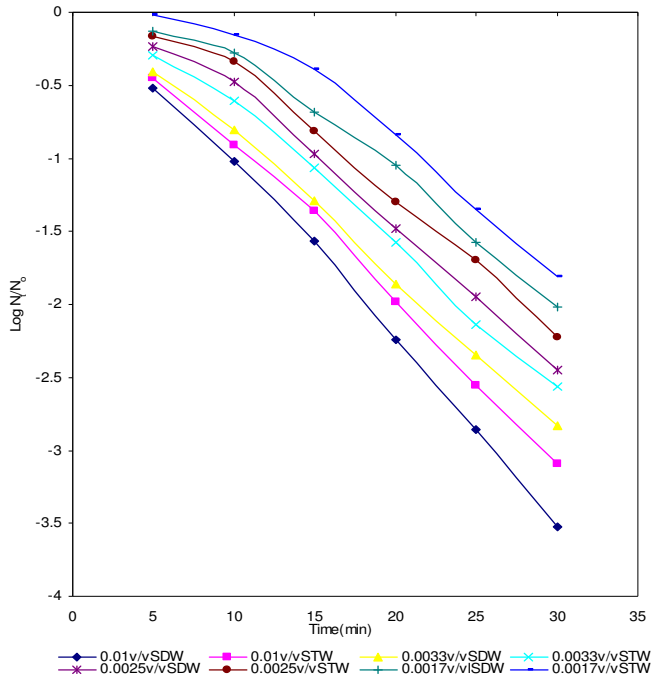

Figure 1a. Effects of use-dilutions of Morigad® (chloroxylenol plus chlorophenol) on the viability of *S. aureus* (S_1) in sterile deionized water (SDW) and sterile tap water (STW) incubated at

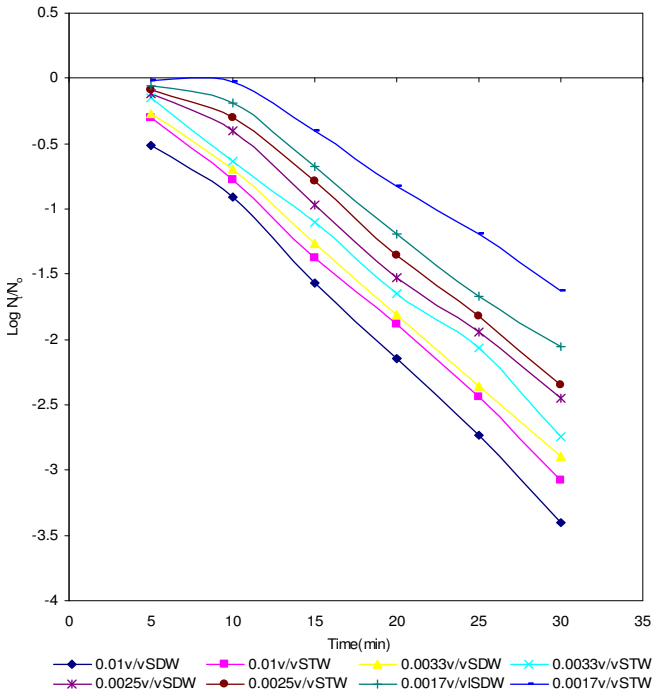

Figure 2a. Effects of use-dilutions of Morigad® (chloroxylenol plus chlorophenol) on the viability of *E. coli* (E_2) in sterile deionized water; (SDW) sterile tap water (STW) incubated at 37°C.

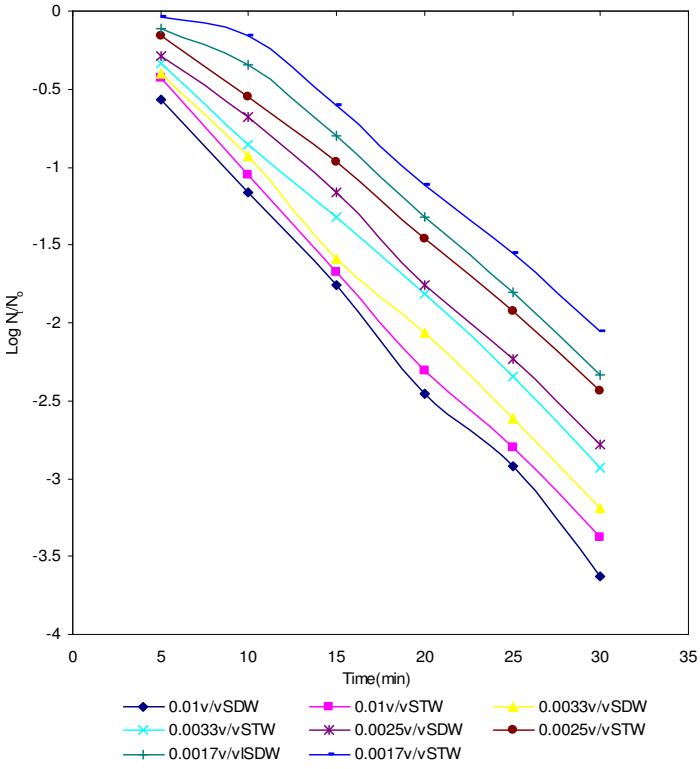

Figure 1b. Effects of use-dilutions of Morigad® (chloroxylenol plus chlorophenol) on the viability of *S. aureus* (S_2) in sterile deionized water; (SDW) sterile tap water (STW) incubated at 37°C.

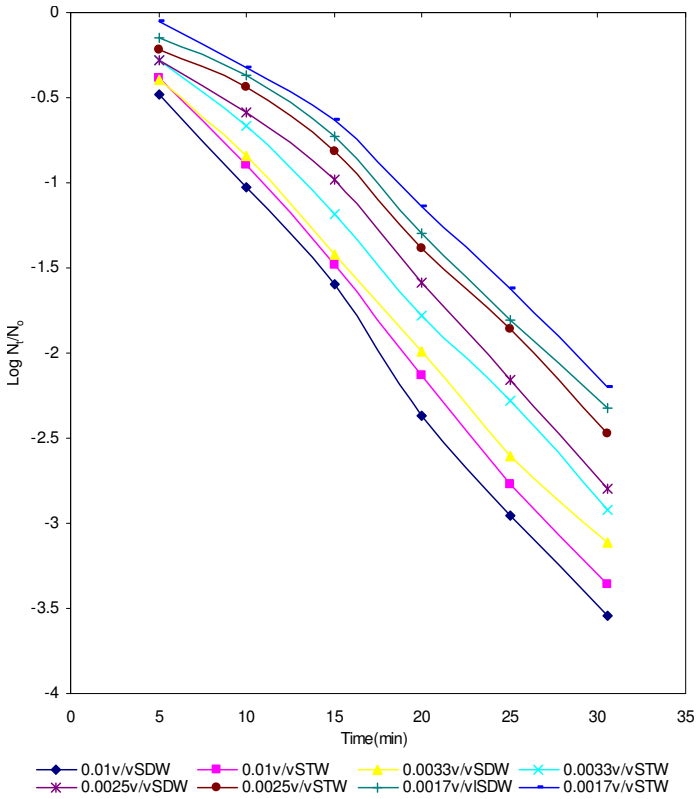

Figure 2b. Effects of use-dilutions of Morigad® (chloroxylenol plus chlorophenol) on the viability of *E. coli* (E_2) in sterile deionized water; (SDW) sterile tap water (STW) incubated at 37°C.

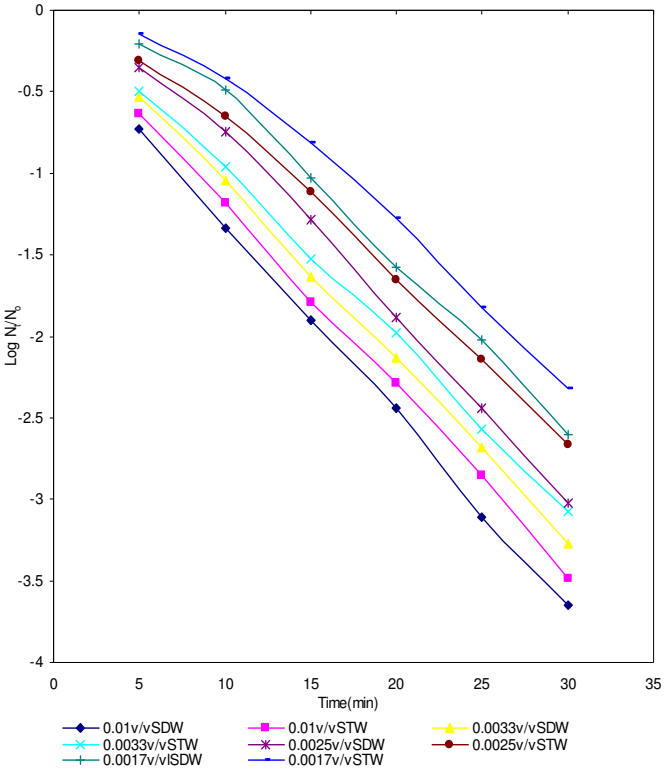

Figure 3a. Effects of use-dilutions of Morigad® (chloroxylenol plus chlorophenol) on the viability of *C. albicans* (C_1) in sterile deionized water; (SDW) sterile tap water (STW) incubated at 37°C.

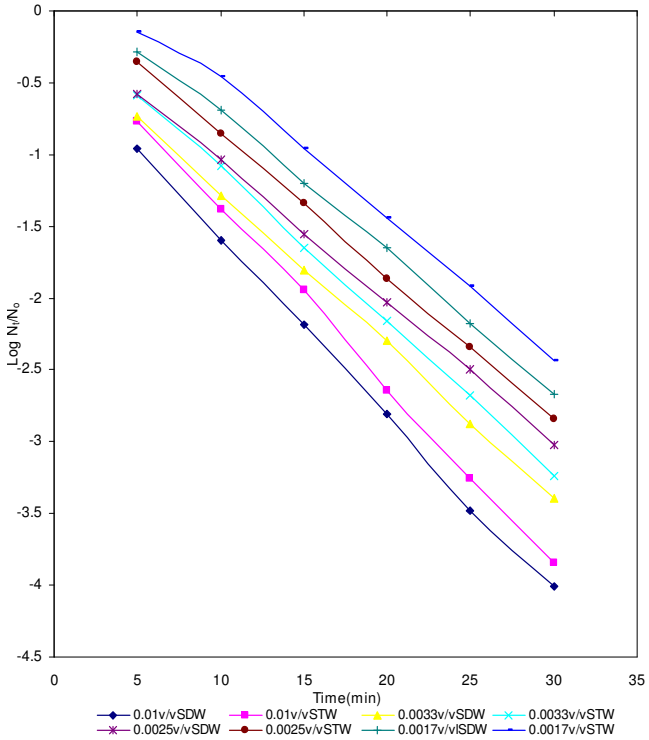

Figure 3b. Effects of use-dilutions of Morigad® (chloroxylenol plus chlorophenol) on the viability of *C. albicans* (C_2) in sterile deioniz-ed water; (SDW) sterile tap water (STW) incubated at 37°C.

shown in Table 3. The slope for 1:100 (0.01 v/v) for *S. aureus* (S_1) was -0.122 in SDW and 0.107 in STW indicating that the rate of the kill was faster in SDW than in STW. For S_2 was, the slope for 1:100 (0.01 v/v) was 0.129 in SDW and -0.125 in STW. The DRT for the 1:100 (0.01 v/v) use dilution for S_1 was 8.20 min in SDW and 0.129 in SDW and -0.125 in STW. The DRT for the 1:100 (0.01 v/v) use dilution for S_1 was 8.20 min in SDW and 7.75 min for S_2. Similarly, the DRT for the 1:100 (0.01 v/v) use dilution in STW for S_1 was 9.34 min and 8.0 min for S_2. This trend was the same for all the organisms and dilutions investigated. The slopes were lower for the test organisms and higher for the control organisms. *E. coli* (E_1 and $_2$) had higher DRT, followed by *S. aureus* (S_1 and $_2$) and then *C. albicans* (C_1 and $_2$). Extrapolation of the curves to the log N_t/N_o axis gives the $\log_{10}$ extra-polation numbers, and the results are shown in Table 4. The result shows the lag times (shoulders) exhibited by each of the graphs. Results revealed that the duration of duration of the lag for S_1 in SDW was 1.63 min and for S_2, 0.63 min. While in STW, the duration was 2.11 for S_1 and 1.13 min for S_2 for the 1:100 (0.01 v/v) use dilutions. For the 1:600 (0.0017 v/v) in SDW, the duration of the lag was 6.08 min for S_1 and 5.10 min for S_2, while in STW, the duration was6.56 min for S_1 and 6.11 for S_2. The lag for E_1 was 2.11, S_1 1.63 and C_1 0.61 min for the 1:100 (0.01 v/v) use dilution. The MIC and MBC values of the disinfectant (Morigad®) for both the cell densities of 1.0 x 10^7 and 1.0 x 10^3 cellsml^{-1} in SDW, STW and 10% rabbit serum are shown in Table 5. Result revealed that for the cell density of 1.0 x 10^7 cellsml^{-1} the MIC values of Morigad® were 0.00022 v/v in SDW, 0.00024 v/v in STW and 0.00026 v/v in 105 rabbit serum, while, the MIC values for the lower cell density of 1.0 x 10^3 cellsml^{-1} were 0.00014 v/v in SDW, 0.00016 v/v in STW and 0.00018 v/v in 10% rabbit serum. The MIC for the lower inoculum density of the control organisms S_2in SDW, STW and 10% rabbit serum were0.00010 v/v, 0.00012 v/v and 0.00014 v/v respectively, while the corresponding values for the higher inoculum density of 1.0 x 10^7 cellsml^{-1} were 0.00016, 0.00018 and 0.00020 v/v respectively. The MIC values of the other four organisms followed a similar trend to that of S_1 and $_2$. Result also showed that the MIC values from the different diluents used were highest for serum, followed by STW, while the lowest values were obtained from SDW. For the organisms investigated, *E. coli* had the highest MIC, followed by *S. aureus*, while *C. albicans* demonstrated the lowest MIC value. Results also revealed that the MBC values were generally higher for all the organisms, but followed a similar pattern to those of MIC.

DISCUSSION

Information generated from questionnaires and Adama-

Table 2. Death rates (K-min^{-1}) of the organisms treated with use-dilutions of Morigad® (chloroxylenol plus chlorophenol) in sterile deionized water (SDW) and sterile tap water (STW).

Conc. (v/v)	Death rate (K-min^{-1})											
	S_1		S_2		E_1		E_2		C_1		C_2	
	SDW	STW	SDW	STW	SDW	STW	SDW	STW	SDW	STW	SDW	STW
0.01	-0.28	-0.25	-0.30	-0.29	-0.27	-0.25	-0.31	-0.28	-0.27	-0.25	-0.28	-0.27
0.0033	-0.25	-0.25	-0.26	-0.22	-0.26	-0.24	-0.27	-0.26	-0.25	-0.23	-0.26	-0.25
0.025	-0.23	-0.22	-0.25	-0.21	-0.26	-0.24	-0.23	-0.22	-0.24	-0.23	-0.23	-0.22
0.0017	-0.18	-0.16	-0.23	-0.22	-0.23	-0.19	-0.21	-0.19	-0.23	-0.20	-0.23	-0.22

S_1 = *S. aureus*; S_2 = *S. aureus*; E_1 = *E. coli*; E_2 = *E. coli*; C_1 = *C. albicans*; C_2 = *C. albicans;* SDW = Sterile deionized water; STW = Sterile tap water.

Table 3. Slope (M) of the curves and decimal reduction time (DRT) of the organisms treated with use–dilutions of Morigad® (chloroxylenol plus chlorophenol) in sterile deionized water (SDW) and sterile tap water (STW).

Conc. (v/v)	DM	NS1		YSB2		FMCE2		FMCE6		NCA3		Mubi-6	
		M	DRT	M	DRT	M	DRT	M	DRT	M	DRT	M	DRT
0.01	SDW	-0.122	8.20	-0.129	7.75	-0.106	9.43	-0.134	7.46	-0.11	9.09	-0.121	8.26
	STW	-0.107	9.34	-0.125	8.00	-0.110	9.09	-0.123	8.70	-0.11	9.09	-0.126	7.94
0.0033	SDW	-0.106	9.43	-0.113	8.85	-0.113	9.01	-0.123	8.70	-0.110	9.09	-0.102	9.80
	STW	-0.097	10.31	-0.096	10.42	-0.102	9.80	-0.115	8.70	-0.101	9.90	-0.108	9.26
0.0025	SDW	-0.100	10.00	-0.108	9.26	-0.113	8.85	-0.100	10.00	-0.114	8.77	-0.100	10.00
	STW	-0.096	10.42	-0.091	10.99	-0.105	9.52	-0.095	10.53	-0.100	10.00	-0.111	9.01
0.0017	SDW	-0.078	12.82	-0.098	10.20	-0.100	10.00	-0.093	10.75	-0.109	9.17	-0.098	10.20
	STW	-0.069	14.49	-0.096	10.42	-0.080	12.50	-0.081	12.35	-0.085	11.77	-0.098	10.20

DM = Dilution medium; S_1 = *S. aureus*; S_2 = *S. aureus*; E_1 = *E. coli*; E_2 = *E. coli*; C_1 = *C. albicans*; C_2 = *C. albicans*; SDW = Sterile deionized water; STW = Sterile tap w

Table 4. Lag and log extrapolation numbers of use-dilution of Morigad® (chloroxylenol plus chlorophenol) against the organisms in sterile deionized water (SDW) and sterile tap water (STW).

Conc (v/v)	DM	Lag and log extrapolation numbers											
		NSI		YSB2		FMCE2		FMCE6		NCA3		Mubi6	
		L	E	L	E	L	E	L	E	L	E	L	E
0.01	SDW	1.63	0.20	0.63	0.10	2.11	0.35	1.13	0.15	0.61	0.05	0.32	0.03
	STW	2.11	0.25	1.13	0.20	2.61	0.40	1.62	0.25	0.61	0.08	0.38	0.05
0.0033	SDW	3.10	0.35	2.11	0.30	3.12	0.55	2.12	0.30	1.11	0.15	0.60	0.08
	STW	4.08	0.65	3.10	0.45	4.10	0.60	3.12	0.45	1.60	0.25	0.91	0.15
0.0025	SDW	4.61	0.50	3.16	0.55	5.11	0.70	3.60	0.50	2.11	0.35	1.30	0.20
	STW	5.10	0.60	4.60	0.68	6.40	0.85	4.10	0.60	2.60	0.40	2.11	0.25
0.0017	SDW	6.08	0.75	5.10	0.75	7.08	0.90	5.10	0.75	3.61	0.55	3.11	0.40
	STW	6.56	0.85	6.11	0.80	7.56	0.95	5.60	0.80	5.09	0.65	4.61	0.55

S_1 = *S. aureus*; S_2 = *S. aureus*; E_1 = *E. coli*; E_2 = *E. coli*; C_1 = *C. albicans*; C_2 = *C. albicans;* SDW = Sterile deionized water; STW = Sterile tap water; L = Lag; E = Log_{10} Extrapolation number; DM= Dilution Medium.

wa State Statistical Year Book (2003) was used in the selection of patients, microorganisms and the disinfectant Morigad®. The use of questionnaires to asses hospital characteristics, study areas and patients for purposes of healthcare investigations have been employed by many researchers (Trick et al., 2004; Kuehnert et al., 2005). The Federal Medical Center Yola selected for this study is an 800 bed tertiary hospital in the State capital. Such large urbam based hospitals have been the centers of many investigations by researchers (Potashc hmacher et al., 1979; Regev-Yachan et al., 2005; Lark et al., 2001; Schroeder et al., 2002).

Table 5. Minimum Bactericidal Concentration values of Morigad® (chloroxylenol plus chlorophenol) against the organisms (Inoculum size $1.0x10^3$ and $1.0x10^7$ cells ml^{-1}).

Organism	Dilution medium	MIC		MBC	
		$1.0x10^3$ cells ml^{-1}	$1.0x10^7$ cells ml^{-1}	$1.0x10^3$ cells ml^{-1}	$1.0x10^7$ cells ml^{-1}
S_1	SDW	0.022	0.030	0.024	0.034
	STW	0.024	0.032	0.026	0.036
	10% Serum	0.026	0.034	0.028	0.038
S_2	SDW	0.016	0.024	0.018	0.026
	STW	0.021	0.026	0.022	0.028
	10% Serum	0.024	0.028	0.026	0.030
E_1	SDW	0.024	0.038	0.026	0.040
	STW	0.028	0.040	0.028	0.042
	10% Serum	0.030	0.042	0.030	0.046
E_2	SDW	0.018	0.026	0.020	0.028
	STW	0.020	0.028	0.022	0.030
	10% Serum	0.022	0.030	0.024	0.032
C_1	SDW	0.020	0.028	0.022	0.030
	STW	0.022	0.030	0.024	0.032
	10% Serum	0.024	0.032	0.026	0.034
C_2	SDW	0.012	0.022	0.014	0.024
	STW	0.014	0.024	0.018	0.026
	10% Serum	0.018	0.026	0.022	0.028

S_1 = *S. aureus*; S_2 = *S. aureus*; E_1 = *E. coli*; E_2 = *E. coli*; C_1 = *C. albicans*; C_2 = *C. albicans*; SDW = Sterile deionized water; STW = Sterile tap water.

The organisms selected for this study were *S. aureus* (S_1 and $_2$), *E. coli* (E_1 and $_2$) and *C. albicans* (C_1 and $_2$). Analysis from questionnaires (results not shown) showed that the predisposing factors associated with hospital acquired infections were diabetes, cancer, HIV, catheter, burns, urinary and respiratory tract infections, preterm neonates with low birth weights, skin and soft tissue infections, septicemia as well as those patients undergoing surgery. The selected organisms have been implicated in nosocomial infections (Schwaber et al., 2004; Scherer et al., 2005). These organisms can also live as commensals but can cause variety of infections as well.

The microbial cells were standardized (calibrated) by determining their growth and generation times by monitoring changes in optical density and viable cell counts. The data produced by turbidimetric and viable cell counts were qualitatively similar. The relationship between turbidimetric and viable cell count data from the basis for standardization of both cultures (Mellefont et al., 2003).

The antimicrobial activity of Morigad® was assessed by performing viable cell counts at 5 min intervals on the surviving microbial population for a period of 30 min (Table 1). The number of cells in both resistant (S_1, E_1 and C_1) and susceptible (S_2, E_2 and C_2) strains were observed to decrease gradually after an initial lag, the duration of which is a function of the concentration of Morigad® used and the type of organism. The number of cells decreased faster in SDW than in STW and also in the susceptible than resistant cells with negative slopes. When a microbial population is subjected to the toxic influence of an agent, the number of cells decreased gradually in such a manner that when the logarithm of the number of cell at any time when plotted against that time falls on a descending straight line with a negative slope (Acheampong et al., 1988). This is referred to as the logarithmic order of death (Esellen and Pflug, 1956) (Table 2). On the other hand, a non-logarithmic order of death had also been reported (Reed et al., 1951; El-Bisi and Ordal, 1956). One characteristic of the logarithmic order of death is that there is a linear relationship between the logarithm of the number of survivors and time. This means that at any time interval a constant proportion of cells loose viability. All the organisms exhibited a uniform response to the Morigad® as shown by the almost straight graphs (Figures 2 - 3). This is an indication that there is no sub population of cells resistant to the Morigad® in the test and control cultures. Extensive work on the mechanism of death in the presence of microbicidal concentrations of phenols and halogenated phenols (including dichloroxylenol and chlorophenol) had been documented and the mode of action of these compounds had been found to be due to their adverse effect on cellular permeability leading to inhibition of enzymes and leakage of intracellular materials out of the cell (Judis, 1962; Alwood and Hugo, 1967; Hugo and Bloomfield, 1971). Thus, the cytoplasmic membrane and its component are considered to be the main site of action of Morigad®.The presence of lag especially in the higher use-dilutions of Morigad® is an

indication that such concentrations had no immediate lethal effects on the cells, probably due to the inadequate effective concentrations of the Morigad® at the target sites. The lag is more pronounced when STW was used as diluent than SDW. The presence of the lag in microbicidal concentrations of toxic agents have been attributed to non uniform distribution of the cells in the suspension as single cells, but were rather grouped as clumps (Meynell and Meynell, 1970; Cove and Holland, 1983).

However, results of this study revealed low values of the lag in high concentration of Morigad when SDW was used as diluent. Variations in use dilutions of Morigad® affected the kinetics of cell death with respect to the length of the lag, the DRT (Table 3), the slope of the graphs and the $\log_{10}$ extrapolation numbers. The relationship between the concentration of Morigad® used and the above parameters are measures of resistance of cells to Morigad®. For complete killing of the cells, a sufficiently high concentration of the Morigad® molecules must be in contact with the organisms for a time greater than the lag prior to exponential order of death. Extrapolation of the graphs to the log N_t/N_o axis and the difference between the intercepts gives the extrapolation number or the multiplicity of the process (Cove and Holland, 1983).

The log extrapolation number gives the number of molecules of the Morigad® required to interact with one cell at that particular concentration in order to cause death (Table 4). The results in this study showed that more molecules of Morigad® are required to produce complete dis-infection especially when STW is used as a diluent in-stead of SDW.

Tap water is reported to contain impurities such as ferrous, calcium, magnesium salts and other trace elements (Wilson and Miles, 1974). These impurities might have interacted with the halogenated phenolic contents in the Morigad® to reduce activity. In this study, the control organisms (S_1, E_1 and C_1) were consistently more susceptible to the use-dilutions of Morigad® than their corresponding index test organisms (S_2, E_2 and C_2). Reports of varying levels of resistance and susceptibility occurring in some species of organisms have earlier been reported and have been attributed variation in lipid build up (Vaczi et al., 1957; Vaczi and Farkas, 1961).

Tap water (STW) was observed to increase the MIC and MBC of Morigad® to values similar to those obtained in the presence of serum (Table 5). Tap water is used in practice to dilute the disinfectant and serum in the part of the likely organic matter encountered in the hospital environment. In the presence of serum, the MIC and MBC values were considerably higher than that without serum. This is not surprising because organic matter have been reported to reduce the activity of most antimicrobial agents (Bean, 1967; Hugo, 1983; Gelinas and Gaulet, 1983; Lynn and Hugo, 1983). The MIC and MBC values were either the same or different with the MBC values higher for most of the time similar to earlier reports (Baldry, 1983). The activity of Morigad is affected by variation in cell density as shown by MIC and MBC results (Table 5).

Morigad was lethal to all the nosocomial isolates studied exhibiting the exponential order of death. The lower use dilutions however, should be used to reconstitute the agent for routine practices.

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