

Full Length Research Paper

# Effect of Dettol® on viability of some microorganisms associated with nosocomial infections

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**The efficacy of the liquid disinfectant Dettol® against nosocomial *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* was investigated. Use dilutions of the disinfectant were not immediately lethal to the microorganisms, with the survival curves exhibiting an initial shoulder before exponential order of death. Tap water adversely affected the death rates and the decimal reduction times (DRT). The usefulness of the product and the implications of the adverse effect of tap water on the activity were discussed.**

**Key words:** Disinfectant, Dettol®, nosocomial infection, survival curves, death rates, decimal reduction times.

## INTRODUCTION

Dettol® is widely used in homes and healthcare settings for various purposes including disinfection of skin, objects and equipments, as well as environmental surfaces. With prior cleaning before application, the number of microorganisms colonizing the skin and surfaces are greatly reduced (Rutala, 1996). The antimicrobial properties of chloroxylenol, the main chemical constituent of Dettol® and other chlorinated phenols have been extensively studied (Hugo and Bloomfield, 1971a). The antimicrobial properties of the disinfectant against some pathogenic bacteria have earlier been reported (Mellefont et al., 2003). There are, however, few or no reports on the activity of this disinfectant on microorganisms causing nosocomial infections. The aims of this study were to investigate the efficacy of Dettol® on some microorganisms associated with nosocomial infections and determine their susceptibilities under use conditions.

## MATERIALS AND METHODS

### Source of microorganisms

The selection of microorganisms was based on analysis of questionnaires previously issued to hospital personnel to determine the most frequent organisms causing nosocomial infections (El Mahmood and Doughari, 2007). The selected microorganisms were

*Staphylococcus aureus*, *Escherichia coli* and *Clostridium albicans*.

Isolation and identification of the organisms was carried out as earlier described (El Mahmood and Doughari, 2007).

The isolated bacteria and fungi were subjected to antimicrobial susceptibility tests as described by Gupta et al. (2004) and Archibald et al. (2004), respectively. Based on susceptibility results obtained, the organisms were grouped into resistant and susceptible strains. Resistant organisms were those that showed stable resistance to more than 3 antibiotics and it is from this group that the test organisms *S. aureus* (SA1), *E. coli* (EC1) and *C. albicans* (CA1) were selected. Those organisms that showed stable susceptibility to all the drugs tested were regarded as susceptible and from these the control strains were *S. aureus* (SA2), *E. coli* (EC2) and *C. albicans* (CA2).

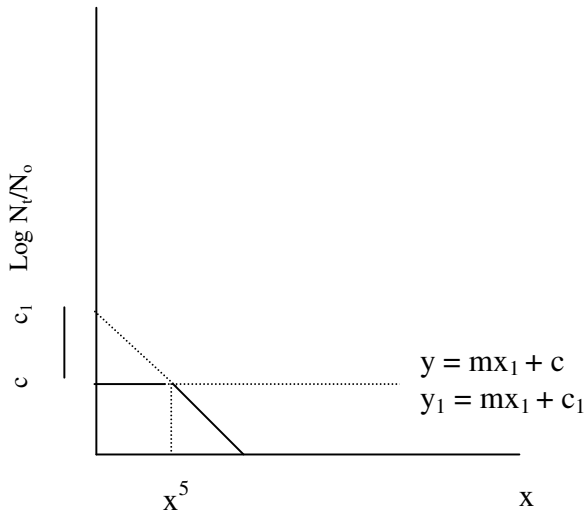
### Source of Dettol®, media and antibiotics

Dettol® (5 L gallon) was purchased from Mamuda Pharmaceutical Stores in Yola, Adamawa State, Nigeria as a commercially formulated product. It consisted of chloroxylenol 4.8% (v/v), oilium pine Aromaticum 9% (v/v), denature spirits 11.3% (v/v), and sapo-vegetalis 5% (v/v). The use dilutions as specified by the manufacturer were 1:20, 1:40 and 3:400. All media and suspending media used were of oxid grade. Antibiotic discs (Optun products) were obtained commercially.

### Determination of survival rates

In order to determine the survival of test organisms in the presence of disinfectant, 1 ml of Dettol® was mixed with 12 ml of sterile deionized water (SDW) in a 50 ml conical flask and 2 ml of *S. aureus* (SA1) with cell density of  $5 \times 10^7$  cell ml<sup>-1</sup> was added to obtain 1:2 (0.05, v/v) use-dilution of Dettol® in 20 ml SDW and he

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**Figure 1.** Analysis of death curves.  $C = C_1 = y = m = m_1$   
 $x_1 = x_5$

content of the flask mixed thoroughly on a whirl mixer (Gallenkamp). 1 ml of the cell suspension was then immediately transferred into a sterile test tube containing 20% v/v tween 80 and 1% v/v soy lecithin and the mixture homogenized on a whirl mixer (Gallenkamp) and allowed to stand for 1 min (Ray et al., 1968 and Russel et al., 1979). Subsequent dilutions of the cell suspension were made in tryptone soy broth (TSB) and 1 ml of the final dilution plated out on nutrient agar plates to obtain countable colonies of 200 - 300 using the pour plate technique at 0 and at 5 min intervals for 30 min. The cultures were then incubated at 37°C for 48 h and the colonies then counted using a Quebec Dark field Colony Counter. This procedure was repeated for each of the other 5 organisms. The experiment was repeated for all the 6 micro-organisms, this time using sterile tap water (STW) in place of sterile deionized water (SDW). The viability of the untreated cultures was also determined at 0 and 5 min intervals for 30 min. Graphs of  $\log N_t/N_0$  versus time were constructed. The experiment was repeated for the other use-dilutions (1: 40 and 3: 400) in STW and SDW.

**Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Dettol®**

The MIC and MBC were determined in nutrient broth using the arithmetic dilution method (Waterworth, 1978) and with the dilutions of the Dettol® in STW, SDW and in the presence of 10% rabbit serum.

**Analysis of death curves**

Linear regression analysis of the viable count data was used to determine the shoulders and exponential death rates. A straight line was fitted to points that appeared to represent then logarithmic phase of death. When the kinetics of cell death exhibit a shoulder, the graph can be represented into two straight lines (Figure 1).

Three important features can be derived from the graph: a) the length of the shoulder  $x_5$  is calculated from the intercept of the straight-line portions of the graph. At the intersect of  $y = y_1$  and  $x = x_1$

$$mx_1 + c = m_1x_5 + C_1$$

$$x_5 = (c_1 - c)/(m - m_1)$$

b). The slope of the killing curve  $m_1$  was used to calculate the decimal reduction time (DRT)

$$DRT = -1/ m_1$$

c). The difference between intercepts  $C_1$  and  $C$  is the extrapolation number (also known as the multiplicity of the process). The death rate ( $k \text{ min}^{-1}$ ) was calculated from the viable cell count data using the equation:

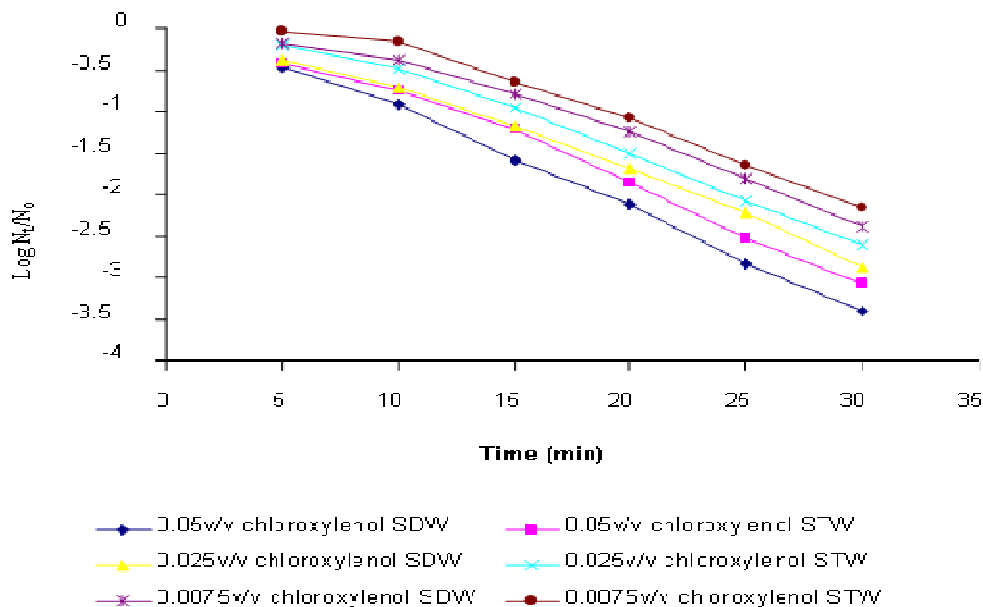
$$K = (t/2.303) \times \log_{10} N_t/N_0$$

**RESULTS**

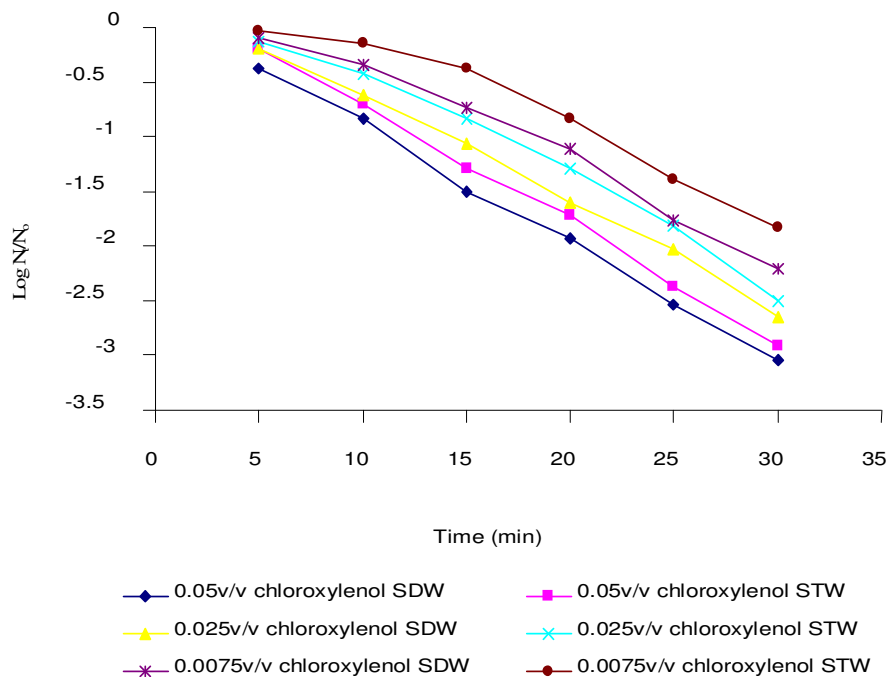
Viable counting techniques was used to determine the number of cells that survived the effects of use-dilutions of Dettol® and the data plotted as  $\log_{10} N_t/N_0$  versus time as in Figure 2 (*S. aureus* SA1), Figure 3 (*S. aureus* SA2), Figure 4 (*E. coli* EC1), Figure 5 (*E. coli* EC2), Figure 6 (*C. albicans* CA1) and Figure 7 (*C. albicans* CA2). For all the organisms there was an overall similarity in the shapes of the curves. There were some initial shoulders before the exponential phases of death depending on which use-dilution of Dettol® and type of organism considered. For each of the organisms, there was little or no decline in the number of cells after 5 min of exposure to Dettol® in both SDW and ATW. The loss of viability was more in SDW than in STW, and in the lower than in the higher used-dilutions (Table 1). However, there was rapid decline in the cell count and after 10 min of treatment, the viability dropped to 12.0% for SA1 decreasing to 0.03% within the next 20% for the 1: 20 use-dilution of Dettol® in SDW. For the higher use dilution 3: 400, after 10 min of contact with the disinfectant, the population of cells decreased to 42.0% and to fewer than 0.40% after 30 min of contact in SDW for *S. aureus* (SA1). A similar trend was recorded with the other 5 organisms (Table 1).

The death rate ( $k \text{ min}^{-1}$ ) of the cultures calculated from the viable count data according to the equation of a unimolecular reaction are given in Table 2. The higher the value of  $k$ , the faster the efficiency of the killing process. The death rates in SDW were higher than that in STW. Thus the death rates ( $k \text{ min}^{-1}$ ) were  $- 0.28$  for 1:20 and  $- 0.20$  for the 3:400 use-dilution of Dettol® against *S. aureus* (SA1) in SDW. While the death rates ( $k \text{ min}^{-1}$ ) for *S. aureus* (SA2) (control) was  $- 0.30$  for 1:20 and  $- 0.23$  for the 3:400 use-dilution of Dettol®. The death rates of the other 4 organisms followed a similar pattern.

Table 3 showed the slopes and the decimal reduction times (DRT) which was time required for 90% reduction in the number of viable cells. The DRT for *S. aureus* (SA1) was 8.26 min for 1:20 and 11.49 min for 3:400 use dilutions in SDW, while for *S. aureus* (SA2), the DRT was 7.63 min for 1:20 and 10 min for 3:400 use-dilution of Dettol®. A similar pattern was recorded for the



**Figure 2.** Effects of use-dilutions of Dettol® (chloroxylenol) on viability of *S. aureus* (SA1) in sterile deionized water (SDW) and sterile tap water (STW) incubated at 37°C.

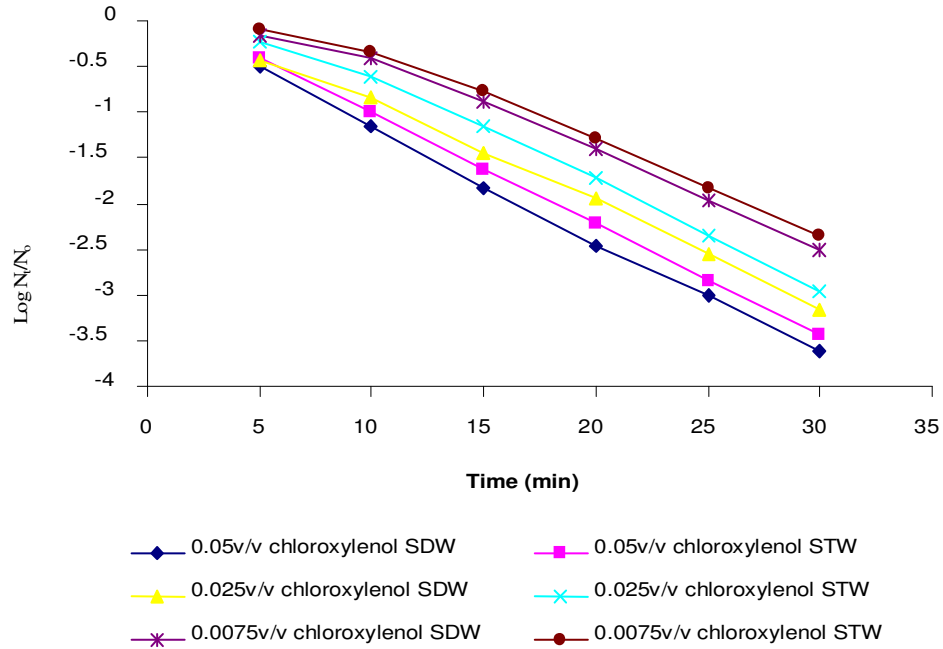


**Figure 3.** Effects of use-dilutions of Dettol® (chloroxylenol) on viability of *S. aureus* (SA2) in sterile deionized water (SDW) and sterile tap water (STW) incubated at 37°C.

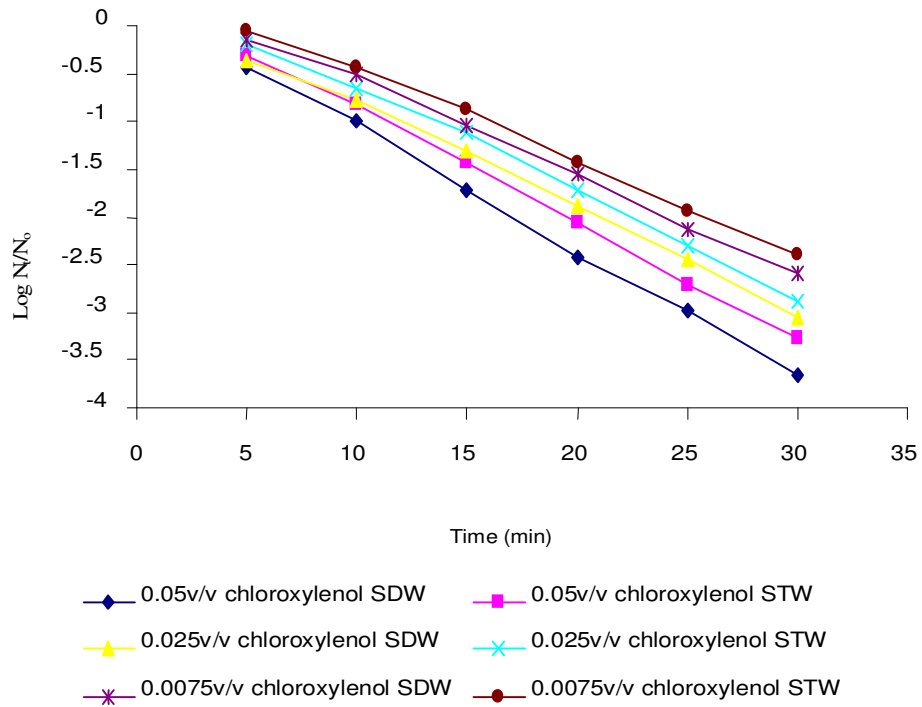
other 4 organisms.

The length of the shoulder (L) and log extrapolation number (E) calculated using the method of Cove and Holland (1983) are shown in Table 4. For *S. aureus* (SA1), L was 1.4 min and E was 0.4 for 1:20, while L

was 3.7 min and E was 0.8 for 3:400 use dilution of Dettol® in SDW. For *S. aureus* (SA2) (control), L was 1.3 min and E 0.2 for 1:20 and L was 3.6 min and E 0.7 for 3:400 use-dilution of Dettol®. This pattern was similar for the other organisms. The L and E are mea-



**Figure 4.** Effects of use-dilutions of Dettol® (chloroxylenol) on viability of *E. coli* (EC1) in sterile deionized water (SDW) and sterile tap water (STW) incubated at 37°C.



**Figure 5.** Effects of use-dilutions of Dettol® (chloroxylenol) on viability of *E. coli* (EC2) in sterile deionized water (SDW) and sterile tap water (STW) incubated at 37 °C

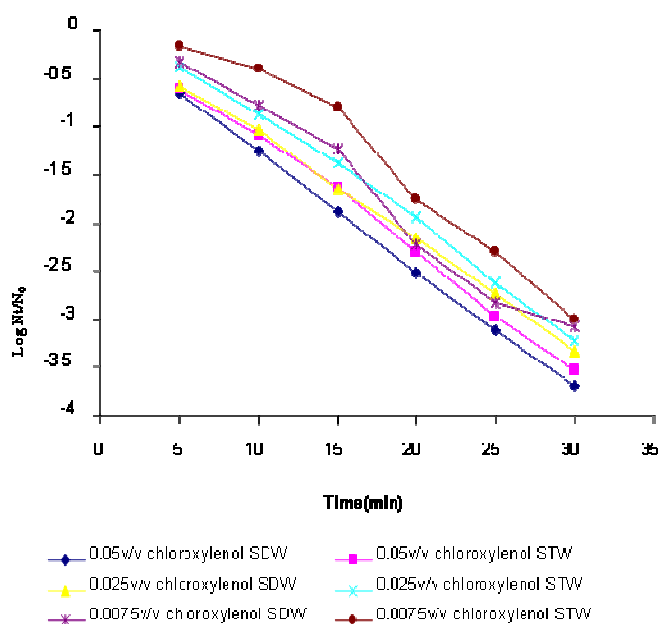
tures of resistance of the cells to the Dettol® and vary with concentration of the disinfectant and type of organism. Extrapolation of the survivor curves to the

$\log_{10} N_t/N_0$  axis gives the extrapolation number or the multiplicity of the process. The difference between the intercepts in the  $\log_{10} N_t/N_0$  axis from the extrapolated

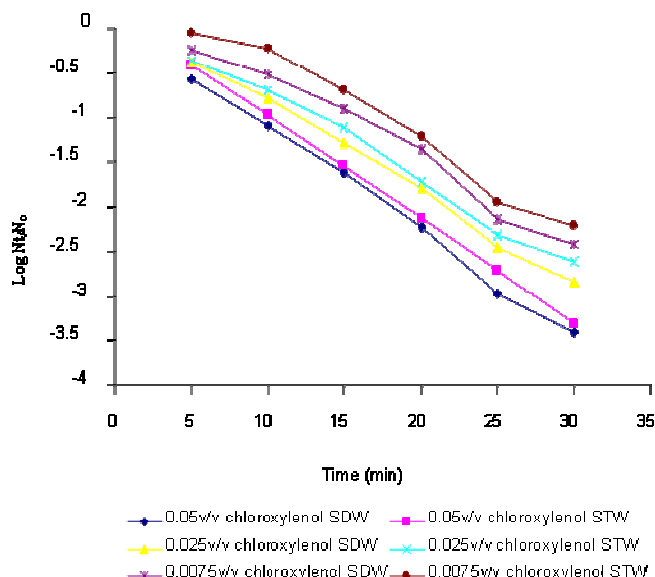
**Table 1.** Percentage of cells surviving after 10 and 30 min exposure to use dilutions of Dettol®.

| Use dilution | Dilution medium | Percentage viable cells/time (min) |      |      |      |      |      |       |      |      |      |      |      |
|--------------|-----------------|------------------------------------|------|------|------|------|------|-------|------|------|------|------|------|
|              |                 | SA1                                |      | SA2  |      | EC1  |      | EC2   |      | CA1  |      | CA2  |      |
|              |                 | 10                                 | 30   | 10   | 30   | 10   | 30   | 10    | 30   | 10   | 30   | 10   | 30   |
| 0.05         | SDW             | 12.0                               | 0.03 | 7.0  | 0.03 | 10.0 | 0.09 | 10.00 | 0.02 | 8.00 | 0.04 | 6.0  | 0.02 |
|              | STW             | 18.0                               | 0.04 | 10.0 | 0.03 | 19.0 | 0.1  | 15.00 | 0.05 | 11.0 | 0.05 | 8.0  | 0.03 |
| 0.025        | SDW             | 19.0                               | 0.10 | 15.0 | 0.07 | 24.0 | 0.20 | 17.00 | 0.09 | 16.0 | 0.1  | 10.0 | 0.05 |
|              | STW             | 33.0                               | 0.20 | 25.0 | 0.10 | 37.0 | 0.30 | 22.00 | 0.10 | 21.0 | 0.3  | 14.0 | 0.06 |
| 0.0075       | SDW             | 42.0                               | 0.40 | 39.0 | 0.30 | 45.0 | 0.60 | 30.00 | 0.30 | 30.0 | 0.4  | 27.0 | 0.20 |
|              | STW             | 69.0                               | 0.70 | 45.0 | 0.40 | 73.0 | 2.00 | 36.00 | 0.40 | 60.0 | 0.6  | 41.0 | 0.50 |

SA1 = *S. aureus* (test organism); SA2 = *S. aureus* (control organism); EC1 = *E. coli* (test organism); EC2 = *E. coli* (control organism); CA1 = *C. albicans* (test organism); CA2 = *C. albicans* (control organism).



**Figure 6.** Effects of use-dilutions of Dettol® (chloroxylenol) on viability of *C. albicans* (CA1) in sterile deionized water (SDW) and sterile tap water (STW) incubated at 37°C.



**Figure 7.** Effects of use-dilutions of Dettol® (chloroxylenol) on the viability for *C. albicans* (CA2) in sterile deionized water (SDW) and sterile tap water (STW) incubated at 37°C.

curve gives the extrapolation number and this, as argued by Cove and Holland (1983), indicates how many molecules of the Dettol® is required to interact with one cell in order to cause death.

The MBC values of Dettol® against the organisms are presented in Table 5. All the 6 strains were killed by Dettol® depending on the inoculum density of the cultures. The MBC range for the inoculum density  $1.0 \times 10^3$  cells ml<sup>-1</sup> was 0.010 - 0.016 (v/v) for *S. aureus* (SA1 and SA2), 0.010 - 0.026 (v/v) for *E. coli* (EC1 and EC2) and 0.008 - 0.018 (v/v) for *C. albicans* (CA1 and CA2). The MBC values were higher for the inoculum density  $1.0 \times 10^7$  cells ml<sup>-1</sup>, but followed a similar pattern in  $1.0 \times 10^3$  cells ml<sup>-1</sup> being higher in 10% rabbit serum followed by STW and the least in SDW.

## DISCUSSION

Counting methods have been used to determine the number of microbial cells that survived the toxic effects of Dettol® at 5 min interval for a period of 30 min. Esselen and Pflug (1956) had reported that when a microbial population is treated with an antimicrobial agent, the number of cells decrease gradually in such a manner that the logarithms of the population of surviving cells at any time when plotted against that time falls in a descending straight line with a negative slope. This is referred to as the logarithmic order of death, meaning that at any given time, a constant number of cells loose viability. On the other hand, a non-logarithmic order of death has also been reported (Reed et al., 1951; El-bisi

**Table 2.** Death rates (k·min<sup>-1</sup>) of the organisms after treatment with use dilutions of Dettol® in SDW and STW.

| Conc (v/v) | Death rate (K·min <sup>-1</sup> ) |       |       |       |       |       |       |       |       |       |       |       |
|------------|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|            | SA1                               |       | SA2   |       | EC1   |       | EC2   |       | CA1   |       | CA2   |       |
|            | SDW                               | STW   | SDW   | STW   | SDW   | STW   | SDW   | STW   | SDW   | STW   | SDW   | STW   |
| 0.05       | -0.28                             | -0.25 | -0.30 | -0.28 | -0.25 | -0.23 | -0.33 | -0.28 | -0.26 | -0.26 | -0.29 | -0.28 |
| 0.025      | -0.24                             | -0.24 | -0.26 | -0.23 | -0.23 | -0.20 | -0.26 | -0.24 | -0.23 | -0.23 | -0.26 | -0.25 |
| 0.0075     | -0.20                             | -0.20 | -0.23 | -0.22 | -0.18 | -0.16 | -0.24 | -0.23 | -0.22 | -0.23 | -0.21 | -0.20 |

SA1 = *S. aureus* (test organism); SA2 = *S. aureus* (control organism); EC1 = *E. coli* (test organism); EC2 = *E. coli* (control organism); CA1 = *C. albicans* (test organism); CA2 = *C. albicans* (control organism).

**Table 3.** Determination of slope (M) of the survival curves and decimal reduction time (DRT) of the organisms treated with use dilutions of chloroxlyenol in sterile deionized water (SDW) and sterile tap water (STW).

| Conc (v/v) | DM  | Decimal reduction time (min) and slope of the curves |       |        |       |       |       |       |       |        |       |        |       |
|------------|-----|--|-------|--------|-------|-------|-------|-------|-------|--------|-------|--------|-------|
|            |     | SA1  |       | SA2    |       | EC1   |       | EC2   |       | CA1    |       | CA2    |       |
|            |     | M  | DRT   | M      | DRT   | M     | DRT   | M     | DRT   | M      | DRT   | M      | DRT   |
| 0.05       | SDW | 0.121  | 8.26  | -0.131 | 7.63  | 0.107 | 9.35  | 0.143 | 6.99  | -0.113 | 8.85  | -0.126 | 7.94  |
|            | STW | 0.110  | 9.09  | -0.127 | 7.87  | 0.099 | 10.1  | 0.123 | 8.13  | -0.115 | 8.7   | -0.120 | 8.33  |
| 0.025      | SDW | 0.099  | 10.1  | -0.111 | 9.01  | 0.100 | 10.00 | 0.114 | 8.77  | -0.100 | 10.00 | -0.112 | 8.93  |
|            | STW | 0.089  | 11.20 | -0.111 | 9.01  | 0.086 | 11.63 | 0.107 | 9.35  | -0.104 | 9.62  | -0.108 | 9.26  |
| 0.0075     | SDW | 0.087  | 11.49 | -0.100 | 10.00 | 0.076 | 13.16 | 0.103 | 9.71  | -0.083 | 12.05 | -0.091 | 10.99 |
|            | STW | 0.085  | 11.77 | -0.093 | 10.75 | 0.071 | 14.08 | 0.099 | 10.10 | -0.098 | 10.20 | -0.088 | 11.36 |

DM = Dilution Medium. M = Slope. DRT = Decimal Reduction Time; - = Not Applicable.

SA1 = *S. aureus* (test organism); SA2 = *S. aureus* (control organism); EC1 = *E. coli* (test organism); EC2 = *E. coli* (control organism); CA1 = *C. albicans* (test organism); CA2 = *C. albicans* (control organism).

**Table 4.** Determination of lag (L) and log extrapolation numbers (E) of chloroxlyenol (D1) against the organisms in sterile deionized water (SDW) and sterile tap water (STW).

| Conc v/v | DM  | Lag and log extrapolation numbers |     |     |     |     |     |     |     |     |     |     |     |
|----------|-----|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|          |     | SA1                               |     | SA2 |     | EC1 |     | EC2 |     | CA1 |     | CA2 |     |
|          |     | L                                 | E   | L   | E   | L   | E   | L   | E   | L   | E   | L   | E   |
| 0.05     | SDW | 1.4                               | 0.4 | 1.3 | 0.2 | 2.3 | 0.3 | 2.0 | 0.2 | 1.1 | 0.2 | 0.6 | 0.1 |
|          | STW | 2.0                               | 0.5 | 2.1 | 0.4 | 3.6 | 0.4 | 2.1 | 0.3 | 1.9 | 0.2 | 1.1 | 0.2 |
| 0.025    | SDW | 2.6                               | 0.5 | 2.2 | 0.5 | 4.6 | 0.5 | 3.1 | 0.3 | 2.1 | 0.3 | 2.2 | 0.3 |
|          | STW | 3.1                               | 0.7 | 3.1 | 0.5 | 5.1 | 0.6 | 3.6 | 0.4 | 2.6 | 0.3 | 2.6 | 0.3 |
| 0.075    | SDW | 3.7                               | 0.8 | 3.6 | 0.7 | 5.7 | 0.6 | 4.1 | 0.6 | 3.6 | 0.4 | 3.6 | 0.4 |
|          | STW | 10.2                              | 0.9 | 7.1 | 0.9 | 7.1 | 0.8 | 7.1 | 0.8 | 6.1 | 0.7 | 5.1 | 0.6 |

SA1 = *S. aureus* (test organism); SA2 = *S. aureus* (control organism); EC1 = *E. coli* (test organism); EC2 = *E. coli* (control organism); CA1 = *C. albicans* (test organism); CA2 = *C. albicans* (control organism).SDW = sterile deionized water; STW = msterile tap water; L = lag; E = log 10 extrapolation number; DM = dilution medium.

and Ordal, 1956). The survivor curves in SDW and STW were qualitatively similar for all the organisms. For *S. aureus* (SA1), the number of cells dropped by 1 log cycle after 10 min of contact and this dropped further by 3 log cycles in the next 20 min of contact for the 1:20 use dilution of Dettol® in SDW. While in STW, for the 1:20 dilution, the number of cells dropped by 1 log cycle after 10 min and 3 log cycles after 30 min contact in the 3:400 use dilution of Dettol® after 10 min. The prepa-

ration of cells dropped by 1 log in SDW and 3 log cycles in the next 20 min. There was no significant decline in the number of cells for the 3:400 use dilution of Dettol® after 10 min in SDW, but this dropped by 3 log cycles after 30 min of contact.

The result showed that Dettol® is a lethal agent against nosocomial microorganisms provided that the recommendations of the manufacturers are adhered to. All the isolates showed a homogenous response to Dettol® as

**Table 5.** Minimum inhibitory concentration values of Dettol® against the test organisms.

| Organism | MBC                        |  |  |
|----------|----------------------------|--|--|
|          | Dilution medium            | 1.0x10 <sup>3</sup> cells ml <sup>-1</sup> | 1.0x10 <sup>7</sup> cells ml <sup>-1</sup> |
| SA1      | SDW                        | 0.016                                      | 0.020                                      |
|          | STW                        | 0.016                                      | 0.022                                      |
|          | 10% Serum                  | 0.020                                      | 0.024                                      |
|          | 95% Ethanol                | NC   | NC   |
|          | 95% Ethanol plus 10% Serum | NC   | NC   |
| SA2      | SDW                        | 0.010                                      | 0.016                                      |
|          | STW                        | 0.012                                      | 0.018                                      |
|          | 10% Serum                  | 0.016                                      | 0.020                                      |
|          | 95% Ethanol                | NC   | NC   |
|          | 95% Ethanol plus 10% Serum | NC   | NC   |
| EC1      | SDW                        | 0.02                                       | 0.026                                      |
|          | STW                        | 0.022                                      | 0.028                                      |
|          | 10% Serum                  | 0.026                                      | 0.030                                      |
|          | 95% Ethanol                | NC   | NC   |
|          | 95% Ethanol plus 10% Serum | NC   | NC   |
| EC2      | SDW                        | 0.010                                      | 0.018                                      |
|          | STW                        | 0.012                                      | 0.018                                      |
|          | 10% Serum                  | 0.016                                      | 0.022                                      |
|          | 95% Ethanol                | NC   | NC   |
|          | 95% Ethanol plus 10% Serum | NC   | NC   |
| CA1      | SDW                        | 0.014                                      | 0.018                                      |
|          | STW                        | 0.016                                      | 0.020                                      |
|          | 10% Serum                  | 0.018                                      | 0.022                                      |
|          | 95% Ethanol                | NC   | NC   |
|          | 95% Ethanol plus 10% Serum | NC   | NC   |
| CA2      | SDW                        | 0.008                                      | 0.014                                      |
|          | STW                        | 0.010                                      | 0.016                                      |
|          | 10% Serum                  | 0.014                                      | 0.018                                      |
|          | 95% Ethanol                | Nc   | NC   |
|          | 95% Ethanol plus 10% Serum | NC   | NC   |

NC = Not carried out; SDW = sterile deionized water; STW = msterile tap water.

SA1 = *S. aureus* (test organism); SA2 = *S. aureus* (control organism); EC1 = *E. coli* (test organism); EC2 = *E. coli* (control organism); CA1 = *C. albicans* (test organism); CA2 = *C. albicans* (control organism).

there was no decrease in killing rates over the period of exponential death. This may imply that there were no subpopulations of cells resistant to use dilution of Dettol® in the cultures tested. All the survivor curves exhibited a shoulder followed by exponential death. Cove and Holland (1983) reported that microorganisms exposed to toxic agents usually show logarithmic death with or without a shoulder and a plot of  $\log N_t/N_0$  against time gives a straight line graph with a negative slope. The length of the shoulder, the slope of the curve which is used to calculate the DRT and the intercepts of the curves are all measurements of resistance of the cells to the agent. Variations in the use dilutions of Dettol® affected the kinetics of cell death with respect to the length of the shoulder, the gradient of the curves and the

DRT. The presence of the shoulder, especially in low-use concentrations of Dettol® is evidence that such concentrations have no immediate lethal effect on the organisms. Meynell and Meynell (1970) had also attributed the presence of the shoulder to the non-uniform distribution of the cells in the suspension as single cells, but were rather grouped clumps. However, the extremely short period of the shoulder at higher concentration of Dettol® in SDW did not support the conclusions of these workers. Similarly, studying the effect of use dilutions of TCP against *P. aeruginosa* and *S. aureus*, no log was observed even with the highest dilution of the antiseptic (Acheampong et al., 1988).

Tap water affected the potency of the Dettol® by increasing the MBC, the length of the shoulder, DRT and

extrapolation numbers. Tap water is known to contain traces of  $Mg^{2+}$ ,  $Fe^{2+}$  and  $Ca^{2+}$  ions and it is possible that these impurities might have reacted with the chloroxylenol component in Dettol® to reduce its effective concentration for activity (Wilson and Miles, 1964; Acheampong et al., 1988). Cove and Holland (1983) suggested that for the complete killing of all the cells, a sufficiently high concentration of an agent must be in contact with the cells in the suspension for a period greater than the shoulder prior to the exponential death (A2). Like phenol, chloroxylenol (Dettol®) is a membrane active agent that are adsorbed into the bacterial cell, and depending on the quantity adsorbed, results in growth inhibition or loss of viability (Hugo and Bloomfield, 1971a). Bactericidal activity results from rapid disruption of the membrane structure and function and the general loss of cytoplasmic constituents from the cell. This membrane damage is irreversible and the cell is thus unable to overcome the loss of essential metabolites (Hugo and Bloomfield, 1971b). Garrett and Brown (1964) had suggested that there was no single concentration of an agent at which all microbial cells in a suspension would be killed instantaneously, and that the process of killing occurs chiefly as a function of time within a range of concentrations. Thus the cytoplasmic membrane and its components are considered to be the main sites of action of chlorinated phenols including Dettol® (Lamikanra and Allwood, 1977).

Extrapolation of the regression lines to the  $\log N_t/N_0$  axis and measurements of the difference between the intercepts gives the log extrapolation number (Cove and Holland, 1983). The log extrapolation number gives the number of the molecules of Dettol® required to interact with one cell at that particular concentration to cause death. The loss of viability of the cultures are more rapid in SDW than in STW and also the death rates are higher in DW than in STW.

The DRT (calculated from slopes of the curves) depended on the use dilution of the Dettol® and also on the type and resistance of the microorganism used. Thus in this study, the test organisms which were multidrug resistant were consistently more resistant to the activity of Dettol® than their corresponding index control microorganisms. The results from this study tend to support the suggestion of a link between antibiotic resistance and resistance to disinfectants. The order of the susceptibility of the pathogens used were *E. coli* (EC1) > *S. aureus* (SA1) > *C. albicans* (CA1) > *E. coli* (EC2) > *S. aureus* (SA2), *C. albicans* (CA2).

The MBC is useful parameter in the assessment of bactericidal activity of an antimicrobial agent. Tap water and serum adversely affected the activity of Dettol®. Microorganisms are rarely found in pure cultures, but enveloped in proteinaceous material. Organic matter like serum has been shown to reduce the activity of an antimicrobial agent by reducing effective concentration of the agent available to microorganisms (Gelinas and

Gauylet 1983 and Lynn and Hugo, 1983). It may also affect the activity by interacting with highly reactive molecules either decomposing them, or combining with them to produce a form less readily adsorbed on the microorganisms (Bean, 1967). Other modes of interference of serum are by adsorption of the agents from solution or by occluding the cells thereby protecting them from the action of the agents (Hugo, 1967). It is possible that the active constituents of Dettol® react with some amino acids and proteins in serum to form inactive products thereby reducing activity and increasing MBC values. Knowledge of the kinetics of loss of viability of microbial population treated with antimicrobial agents had been used to predict and control disinfection and sterilization procedures (Hugo, 1967). The determination of the MBC, the shoulder prior to exponential phase of death, the slope of the death curves, the death rates, the DRT and the extrapolation number had made it possible to compare the resistance of one particular organism at different use dilution of Dettol®. Thus in this study, *E. coli* was more resistant than *S. aureus* which in turn was more resistant than the control organism.

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